University of Alberta

The Neurochemical Effects of Lithium and Valproate

By

Tina Marie O'Donnell ©

A thesis
submitted to the Faculty of Graduate Studies and
Research in partial fulfillment of the requirements
for the degree of Master of Science

Department of Psychiatry

Edmonton, Alberta

Spring 2001

National Library
of Canada

Acquisitions and
Bibliographic Services

395 Wellington Street
Ottawa ON K1A 0N4
Canada

Bibliothèque nationale
du Canada

Acquisitions et
services bibliographiques

395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence

Our file Notre référence

The author has granted a non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of this thesis in microform, paper or electronic formats.

L'auteur a accordé une licence non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de cette thèse sous la forme de microfiche/film, de reproduction sur papier ou sur format électronique.

The author retains ownership of the copyright in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

L'auteur conserve la propriété du droit d'auteur qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

0-612-60483-7

Canada

University of Alberta

Library Release Form

Name of Author: Tina Marie O'Donnell

Title of Thesis: The Neurochemical Effects of Lithium and Valproate

Degree: Master of Science

Year this Degree Granted: 2001

Permission is hereby granted to the University of Alberta Library to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves all other publication and other rights in association with the copyright in the thesis, and except as herein before provided, neither the thesis nor any substantial portion thereof may be printed or otherwise reproduced in any material form whatever without the author's prior written permission.

Tina O'Donnell

Box 831
Bow Island, AB
T0K 0G0
Canada

Date: Dec 21/2000

University of Alberta

Faculty of Graduate Studies and Research

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled "The Neurochemical Effects of Lithium and Valproate" submitted by Tina Marie O'Donnell in partial fulfillment of the requirements for the degree of Master of Science

P. Silverstone
Peter Silverstone - supervisor

Glen Baker – co-supervisor

Andrew Greenshaw

Susan MJ Dunn
Susan Dunn

1/Nov/2000
Date of approval

Dedication

This thesis is dedicated to my parents, Blaine and Donna, for all their love and support throughout the years.

Abstract

The effects of lithium and valproate, two commonly used mood stabilizers in the treatment of Bipolar Disorder, on concentrations of *myo*-inositol, inositol monophosphates, and other neurotransmitters were examined using high-resolution nuclear magnetic resonance (NMR) spectroscopy. The concentrations of several amino acids were examined in whole rat brain using NMR and high performance liquid chromatography (HPLC). Both lithium and valproate caused a decrease in the concentrations of *myo*-inositol and an increase in the concentrations of inositol monophosphates. Lithium and valproate decreased the concentrations of aspartate, creatine, glutamate, N-acetylaspartate, and taurine. Only valproate decreased concentrations of GABA and alanine. These results suggest that lithium and sodium valproate may share effects in the treatment of Bipolar Disorder via actions on the PI-cycle and/or amino acid neurotransmitters.

Acknowledgements

I would first like to thank Dr. Peter Silverstone and Dr. Glen Baker for their support and encouragement throughout my program. Their patience and guidance was much appreciated. I would also like to thank Dr. Susan Rotzinger who always set a good example and played an instrumental role in my education.

Grant support was provided by the Medical Research Council of Canada, Canadian Psychiatric Research Foundation, and Alberta Heritage Foundation for Medical Research.

I would also like to thank a number of people who provided exceptional technical support: Ms. Gail Rauw, Ms. Jordyce van Muyden, Mr. Rick Strel, Ms. Veronique Tanay, and Ms. Emily Gordon. Their patience and willingness to teach provided a truly valuable learning experience.

Finally, I would like to thank my fellow graduate students, especially Ms. Michele Ulrich and Ms. Sheila Asghar, for their moral support and friendship.

Table of Contents

Introduction……1

A. General Introduction……1

B. Use of *In Vivo* MRS in Psychiatry……5

  B.1. Introduction to *In Vivo* MRS……5

  B.2. ^{31}P MRS……7

  B.3. ^{1}H MRS……9

C. Effects of Lithium and Valproate on the PI-cycle……12

  C.1. Introduction……12

  C.2. The PI-Cycle……14

  C.3. Lithium and the Inositol-Depletion Hypothesis……16

    C.3.1. Animal Evidence for the Inositol-Depletion Hypothesis……18

    C.3.2. Stimulation with Agonists and the Use of Radiolabelled Substrates……20

    C.3.3. Effects of Lithium on G-proteins in Animals……24

  C.4. Valproate and the PI-cycle……25

  C.5. Problems With Animal Data……26

C.6. Examination of the Inositol-Depletion Hypothesis in Human Subjects....29

C.6.1. Peripheral, CSF and Post-Mortem Measures of the Inositol-Depletion Hypothesis....29

C.6.2. ^{1}H MRS Measures of *Myo*-inositol....32

C.6.3. ^{31}P MRS Measures of Inositol Monophosphates....35

C.6.4. Problems With *In Vivo* Human MRS Studies....38

C.7. Summary and Implications of Research Related to the Inositol-Depletion Hypothesis....42

D. Effects of Lithium and Valproate on Amino Acid Neurotransmitters....44

D.1. Introduction....44

D.2. GABA....44

D.3. Glutamate....47

D.4. Other Amino Acid Neurotransmitters....48

D.5. Summary and Analysis of Amino Acid Studies....50

E. *d*-Amphetamine as a Model of Mania....51

F. Hypotheses and Aim of Study....53

F.1. Part I: The Use of NMR/MR Spectroscopy to Study the Inositol-Depletion Hypothesis in Rat Brain Extracts……………..53

F.2. Part II: The Use of NMR Spectroscopy to Study the Effects of Lithium and Valproate on Amino Acid Concentrations……………………………………………………..54

Materials and Methods………………………..………………………55

A. Methods 1: Measurement of Brain Neurochemicals in Whole Brain Extracts of Rats Chronically Treated with Lithium or Sodium Valproate Using NMR Spectroscopy……………………….………....55

A.1. Chemicals..………………………..…………………………..…..…55

A.2. Drug Administration and Sacrifice of Animals……………….....56

A.3. Preparation of Extracts for NMR Analysis…………………..….57

A.4. Acquisition of ^{31}P and ^{1}H NMR Spectra……………….………..58

A.5. Spectral and Statistical Analysis…………………………….…..58

B. Methods 2: Measurements of Amino Acid Neurotransmitter Concentrations using HPLC...…………………………………..………60

B.1. Chemicals……………………….……………………………….60

B.2. Drug Administration and Sacrifice of Animals……..………...60

B.3. Preparation of Brains for HPLC Analysis of Amino Acids……..…………………………………..…………62

B.4. HPLC Analysis……………………………………………62
  B.4.1. OPA Reagent……………………………………………62
  B.4.2. HPLC Set-up……………………………………………63
  B.4.3. Standard Solutions and Determination of
    Amino Acid Concentrations……………………………66

Results………………………………………………………67

A. Study 1: The Effects of Lithium and Valproate on the Concentrations
  of *Myo*-inositol and the Inositol Monophosphates Using
  High-Resolution NMR Spectroscopy………………………67
  A.1. ^{1}H NMR Spectroscopy…………………………………67
  A.2. ^{31}P NMR Spectroscopy………………………………71
B. Study 2: The Use of NMR Spectroscopy and HPLC to Study the
C.
  Effects of Lithium and Valproate on Amino Acid
  Neurotransmitter Concentrations…………………………76
  B.1. ^{1}H NMR Spectroscopy Results…………………………76
  B.2. HPLC Results……………………………………………77

Discussion..86

A. Part I: The Use of NMR/MR Spectroscopy to Study the Inositol-Depletion Hypothesis in Brain Extracts 86

A.1. Effects of Lithium and Valproate on Myo-inositol and Inositol Monophosphates...86

A.2. The Effects of *d*-Amphetamine on the PI-cycle.....................90

A.3. Quantification Issues in Examining the Inositol-Depletion Hypothesis..90

A.3.1. Ratio Data Expression...91

A.3.2. Co-resonance of Compounds and the Inositol-Depletion Hypothesis...............................95

A.4. Part I: Summary of Findings...96

B. Part II: The Use of NMR Spectroscopy to Study the Effects of Lithium and Valproate on Amino Acid Concentrations................. 98

B.1. The Use of NMR Spectroscopy to Measure Amino Acids...98

B.2. GABA...99

B.3. Glutamate..101

B.4. Aspartate..102

B.5. Taurine..103

B.6. Alanine...104

B.7. Effects of *d*-Amphetamine on Amino Acid Neurotransmitters...104

B.8. Part II: Summary of Findings...105

C. Overall Conclusion...106

References...108

Appendix I...125

A. Calculation of Brain Metabolite Concentrations Using ^{1}H NMR...125

B. Calculation of Brain Metabolite Concentrations Using ^{31}P NMR...126

Appendix II...127

A. HPLC Calibration Plots for Amino Acid Concentrations...127

List of Tables

Table 1 Radiolabelling/Agonist Stimulation Studies of the PI-cycle...22

Table 2 Chemicals Used in Method 1.......................................55

Table 3 Chemicals Used in Method 2.......................................60

Table 4 Concentrations of Amino Acids in Standard Solutions...........66

Table 5 Concentrations of Metabolites in Rat Brain Following Chronic Treatment with Lithium or Valproate.........................75

Table 6 Comparison of NMR Spectroscopy and HPLC in Quantifying Amino Acid Neurotransmitters..................85

List of Figures

Figure 1 Human *In Vivo* ^{31}P MRS Spectrum of Temporal Lobe Brain Tissue........7

Figure 2 Human *In Vivo* ^{1}H MRS Spectrum of Temporal Lobe Brain Tissue........11

Figure 3 Phosphoinositol Cycle (PI-cycle)........17

Figure 4 HPLC Chromatograph of Amino Acids Isolated From a Control Rat Brain........65

Figure 5 500Mz ^{1}H NMR Spectrum of Rat Brain Extract Between 3.51 and 3.66 ppm........68

Figure 6 Brain *Myo*-inositol Concentrations Following Chronic Lithium and Valproate Treatment and Acute *d*-Amphetamine Administration to Rats........69

Figure 7 Brain Cr+PCr Concentrations Following Chronic Lithium and Valproate Treatment and Acute *d*-Amphetamine Administration to Rats........70

Figure 8 Brain NAA Concentrations Following Chronic Lithium and Valproate Treatment and Acute *d*-Amphetamine Administration to Rats........71

Figure 9 202.3MHz ^{31}P NMR Spectrum of Rat Brain Extract Focussed Between 3.00 and 7.25 ppm..........................72

Figure 10 Brain IP_1+PE Concentrations Following Chronic Lithium and Valproate Treatment or Acute *d*-Amphetamine Administration to Rats..73

Figure 11 Rat Brain Concentrations of GABA Following Chronic Treatment with Saline, Lithium, or Valproate as Measured by High-resolution NMR Spectroscopy...............79

Figure 12 Rat Brain Concentrations of GABA Following Chronic Treatment with Saline, Lithium, or Valproate as Measured by HPLC...79

Figure 13 Rat Brain Concentrations of Glutamate Following Chronic Treatment with Saline, Lithium, or Valproate as Measured by High-resolution NMR Spectroscopy...............80

Figure 14 Rat Brain Concentrations of Glutamate Following Chronic Treatment with Saline, Lithium, or Valproate as Measured by HPLC...80

Figure 15 Rat Brain Concentrations of Glycine Following Chronic Treatment with Saline, Lithium, or Valproate as Measured by High-resolution NMR Spectroscopy..........................81

Figure 16 Rat Brain Concentrations of Glycine Following Chronic Treatment with Saline, Lithium, or Valproate as Measured by HPLC.....81

Figure 17 Rat Brain Concentrations of Aspartate Following Chronic Treatment with Saline, Lithium, or Valproate as Measured

Figure 18 Rat Brain Concentrations of Aspartate Following Chronic Treatment with Saline, Lithium, or Valproate as Measured by HPLC.....82

Figure 19 Rat Brain Concentrations of Alanine Following Chronic Treatment with Saline, Lithium, or Valproate as Measured by High-resolution NMR Spectroscopy.....83

Figure 20 Rat Brain Concentrations of Alanine Following Chronic Treatment with Saline, Lithium, or Valproate as Measured by HPLC.....83

Figure 21 Rat Brain Concentrations of Taurine Following Chronic Treatment with Saline, Lithium, or Valproate as Measured by High-resolution NMR spectroscopy.....84

Figure 22 Rat Brain Concentrations of Taurine Following Chronic Treatment with Saline, Lithium, or Valproate as Measured by HPLC.....84

Figure 23 *Myo*-inositol/(Cr+PCr) Ratios Following Chronic Lithium or Sodium Valproate Administration..................................93

Figure 24 HPLC Calibration Plot for GABA Concentrations..............128

Figure 25 HPLC Calibration Plot for Glutamate Concentrations..........129

Figure 26 HPLC Calibration Plot for Aspartate Concentrations..........130

Figure 27 HPLC Calibration Plot for Glycine Concentrations............131

Figure 28 HPLC Calibration Plot for Alanine Concentrations............132

Figure 29 HPLC Calibration Plot for Taurine Concentrations............133

Abbreviations

5-HT	5-hydroxytryptamine
$[^{35}S]GTP\gamma S$	$[^{35}S]$guanosine-5'-O(3-gamma-thio)triphosphate
ANOVA	analysis of variance
AP-1	activator protein 1
ATP	adenosine triphosphate
b.i.d	twice a day
cAMP	cyclic adenosine monophosphate
CDG-DG	cytidine diphosphate diacylglycerol
Cr	creatine
CSF	cerebrospinal fluid
D_2O	deuterium oxide
DAG	*sn*-1,2-diacylglycerol
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, 4th Ed.
DSS	methylenediphosphonic acid trisodium salt tetrahydrate
fMLP	formylmethionylleucylphenylalanine
G6P	glucose-6-phosphate
GABA	γ-aminobutyric acid

GABA-T	GABA transaminase
GAD	glutamic acid decarboxylase
GDP	guanine diphosphate
GPC	glycerophosphocholine
GPE	glycerophophoethanolamine
GTP	guanine triphosphate
HPLC	high performance liquid chromatography
i.c.v.	intracerebroventricular
IMPase	inositol monophosphatase
Ins[1,4,5]P_3	inositol 1,4,5-triphosphate
Ins[1]P	inositol-1-phosphate
Ins[3]P	inositol-3-phosphate
Ins[4]P	inositol-4-phosphate
ip	intraperitoneal
MeOH	methanol
MR	magnetic resonance
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
NAA	N-acetylaspartate
NaF	sodium fluoride

NaOH	sodium hydroxide
NMR	nuclear magnetic resonance
NTP	nucleotide triphosphate
OPT	o-phthalaldehyde
PA	phosphatidic acid
PC	phosphocholine
PCr	phosphocreatine
PDE	phosphodiesters
PERCH	Peak Research
PE	phosphoethanolamine
P_I	inorganic phosphate
PI	phosphatidylinositol
PI-cycle	phosphatidylinositol cycle
PIP_2	phosphatidylinositol 4,5-bisphosphate
PLC	phospholipase C
PME	phosphomonoesters
ppm	parts per million
sc	subcutaneous
SEM	standard error measurement
TCA	tricarboxylic acid cycle

THF	tetrahydrofuran
TSP	sodium 3-trimethylsilyl [2,2,3,3, -^{2}H] propionate

Introduction

A. General Introduction

Bipolar Affective Disorder, otherwise known as manic-depressive illness, is a serious psychiatric illness occurring in approximately 1 percent of the population. Those afflicted with Bipolar Disorder suffer from mood fluctuations between manic, depressed, and euthymic states (DSM-IV) [American Psychiatric Association, 1994], and this mood instability causes disruptions in social, familial, and occupational functioning, leading to significant distress for both the patient and family.

Depending on the severity of the manic episode(s), patients with Bipolar Affective Disorder are further classified as Bipolar I or Bipolar II as outlined fully in the DSM-IV [American Psychiatric Association, 1994]. Bipolar I patients have manic episodes with symptoms such as inflated self-esteem, decreased need for sleep, flight of ideas, distractibility, an increase in goal-directed behavior, and an increased participation in high-risk activities, leading to a disruption in the normal everyday functioning of the patient. Bipolar II patients, however, have hypomanic episodes where both the length and severity of the manic episode is not extreme enough to cause a marked disruption in the functioning of the patient.

While the pathology underlying Bipolar Disorder has not been clearly established, a number of pharmaceutical agents have been effective in both treating and suppressing the clinical symptoms of both mania and depression. Lithium has remained a mainstay for the treatment of Bipolar Disorder, but anti-epileptic medications such as sodium valproate, carbamazepine, and lamotrigine are now commonly used as mood stabilizers [Post et al., 1998]. In addition, a number of other classes of drugs are used as adjunctive therapies to treat symptoms of Bipolar Disorder. These include antidepressants [Yatham et al., 1997], anti-psychotics [Ghaemi and Goodwin, 1999], and calcium channel blockers [Dubovsky, 1993].

The use of lithium for the treatment of Bipolar Disorder was first introduced in 1949 by John Cade who observed a marked improvement in the manic symptoms of a bipolar patient after chronic administration of lithium citrate [Cade, 1949]. Five decades later, lithium remains an important mood stabilizer for both the alleviation of manic symptoms and the prevention of subsequent manic and depressive episodes [Baastrup et al, 1970; Atack, 2000].

Unfortunately, the use of lithium in bipolar patients leads to a number of critical problems and treatment issues. The side effect profile of lithium at therapeutic plasma concentrations can lead to drug intolerance and thus, discontinuation of treatment. Because of its narrow therapeutic window, lithium toxicity can become a problem in some patients. Early symptoms of lithium toxicity include coarse tremor, dysarthria, and ataxia while later signs and symptoms include impaired consciousness, muscular fasciculations, myoclonus, seizures, and coma [Kaplan and Sadock, 1998].

A recent study by Bowden and colleagues [1994] found that lithium and valproate are equally effective in the treatment of acute manic episodes. Valproate has also been found to be effective in the long-term prophylaxis of Bipolar Disorder [Lambert et al., 1975; Lambert, 1984] and is better tolerated than lithium [Bowden et al., 1994; Lambert and Venaud, 1992]. Additionally, valproate may be more effective than lithium in the treatment of rapid cycling patients, adolescents, and the elderly, in addition to those with mixed episodes or concurrent substance abuse disorders [Bowden, 2000].

The therapeutic mechanisms of action of both lithium and valproate are poorly understood, but our knowledge of these compounds is continually evolving due to technological advances in fields such as molecular biology and magnetic resonance spectroscopy (MRS). Lithium's mechanism of action is generally associated with its effects on the phosphatidylinositol cycle (PI-cycle) where it inhibits the enzyme inositol monophosphatase (IMPase), leading to an accumulation of inositol monophosphates and a depletion of their corresponding metabolic product, *myo*-inositol [Berridge et al., 1982]. Valproate's mechanism of action has, however, been linked to its effects on amino acid neurotransmitters like γ-aminobutyric acid (GABA) [Emrich et al., 1980; Petty, 1996]. MRS provides scientists with a non-invasive tool to explore the effects of lithium and valproate on concentrations of *myo*-inositol, inositol monophosphates, and amino acids in healthy and affected human brain *in vivo*. Thus, MRS may provide information regarding the effects of mood stabilizers used for the treatment of Bipolar Disorder on neurochemical systems in functioning human brain.

B. Use of *In Vivo* MRS in Psychiatry

B.1. Introduction to *In Vivo* MRS

MRS is a unique, non-invasive technique that allows researchers to study biochemistry and metabolism *in vivo*, in conjunction with structural information provided by magnetic resonance imaging (MRI). In essence, MRI can be used to visualize small brain regions of interest, and using MRS, the biochemistry of highly mobile molecules in specific brain regions can be examined.

The underlying physical principles of MRS and MRI are from nuclear magnetic resonance (NMR) spectroscopy, which was initially used as a quantitative and qualitative tool for the analysis of small homogenous samples. Briefly, atomic nuclei, which possess a magnetic moment, are first aligned with a large, static magnetic field (Farrar, 1990). While *in vitro* or *ex vivo* NMR experiments may use magnets with field strengths as high as 18 tesla (T), *in vivo* human MRS experiments use low-field magnets (i.e. 1-4T) leading to significantly decreased sensitivity and lower resolution in the acquired spectra (Allen, 1990). Following alignment with a static magnet, a rotating magnetic field or radiofrequency pulse is used to excite the nuclei. Each nucleus then relaxes back to its resting state (Farrar, 1990). Assuming molecules containing the nuclei are in a heterogeneous environment (e.g. functioning human brain), each nucleus will interact with various internal and external magnetic fields in addition to surrounding electronic magnetic fields and thermal phonons (Allen, 1990). Thus, each signal observed in an *in vivo* MRS spectrum is characteristic of the frequency at which a given nucleus resonates back to its resting state under the influence of these factors. The frequency at which relaxation occurs for a given proton or set of protons is called the chemical shift. Thus, each signal is observed on a frequency scale at characteristic chemical shifts.

A number of nuclei known to be important in physiological systems can be detected using MRS. The two mostly commonly studied nuclei in *in vivo* human MRS studies are ^{1}H and ^{31}P due to their high natural abundances of 99.98% and 100% respectively. However, the sensitivity of ^{31}P is 8.3% relative to that of ^{1}H in MR spectra [Cady, 1990]. While ^{1}H MR spectra yield information about a number of biological compounds containing ^{1}H nuclei, ^{31}P spectra provide information about tissue energetics, phospholipid metabolism, and pH. Other detectable isotopes important in psychiatry include ^{13}C, ^{7}Li and ^{19}F. Lithium salts are commonly used to treat Bipolar

Disorder, and thus the detection of lithium is important for the measurement of tissue levels in the brain.

B.2. ^{31}P MRS

Using ^{31}P MR spectroscopy, it is possible to detect a number of phosphorus compounds *in vivo*, as observed in Figure 1.

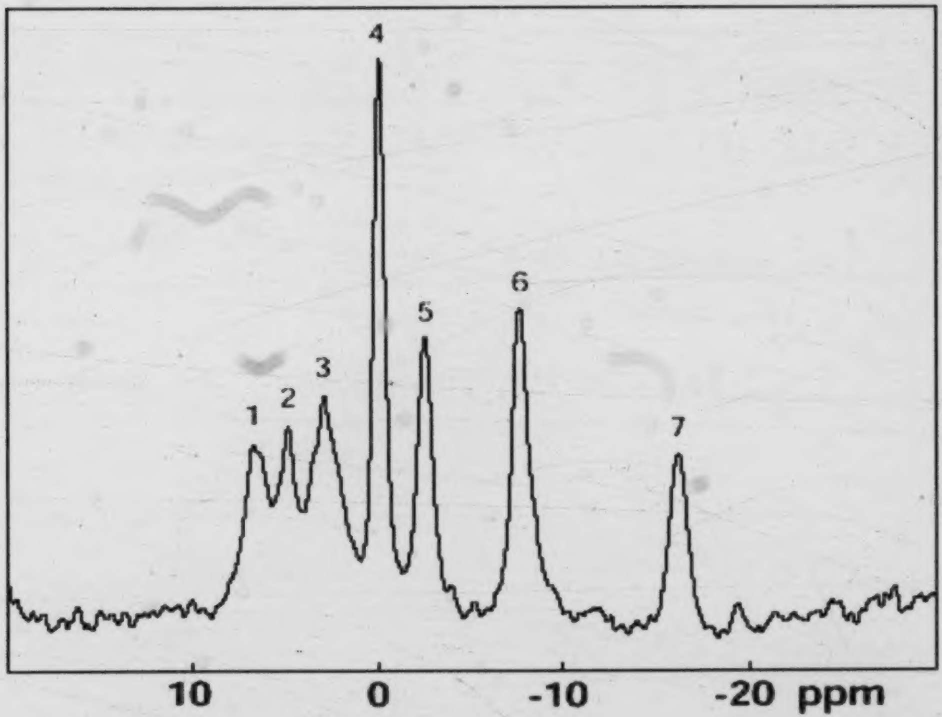

Figure 1: Human In Vivo ^{31}P MRS Spectrum of Temporal Lobe Brain Tissue. Peak assignments are as follows: 1 = PME (phosphomonoesters); 2 = P_i (inorganic phosphate); 3 = PDE (phosphodiesters); 4 = PCr (phosphocreatine); 5 = γ-ATP; 6 = α-ATP; 7 = β-ATP

The ^{31}P phosphomonoester peak (PME) consists of phosphoethanolamine (PE) [Gyulai et al., 1984; Preece et al., 1992], phosphocholine (PC), and sugar phosphates like the inositol monophosphates and glucose 6-phophate (G6P) [Preece et al., 1992]. Unfortunately, due to low magnet field strengths used in *in vivo* MRS, it is not possible to resolve the signal contributions from each individual compound. Other signals of interest observed in ^{31}P spectra include peaks from inorganic phosphate (P_i), phosphodiesters (PDE), phosphocreatine (PCr), and α-, β-, and λ-nucleotide triphosphates (NTP) which mainly consist of signals from adenosine triphosphate (ATP).

The PME peak is of primary importance for the study of Bipolar Disorder, where it is thought that lithium increases the amount of brain inositol monophosphates due to an inhibition of inositol monophosphatase (IMPase) [Hallcher and Sherman, 1980; Naccarato et al, 1974; Nahorski et al, 1991]. However, because the inositol monophosphates are contained in a peak with a number of other compounds, there is some controversy over whether observed changes in this peak are reflective of concentration changes in the inositol monophosphates.

The expression of data obtained from ^{31}P MRS has been inconsistent as no clear protocol has been established. Normally, ratio data are presented where the numerator is the signal from the peak of interest and the denominator is a signal which is thought to remain stable under most circumstances. For the ratio data to be meaningful, the internal reference used as the denominator must not significantly vary between healthy controls and patients and must remain stable under conditions such as environmental changes, drug treatment, and changes in psychiatric state. In the past, researchers have used denominators such as total phosphorus, PCr, and β-ATP. However, changes in these compounds due to drug treatment or a psychiatric condition have not been studied and thus the use of internal references has not been validated.

B.3. ^{1}H MRS

In vivo ^{1}H MRS has gained attention in the study of Bipolar Disorder due to its ability to measure brain concentrations of *myo*-inositol. However, the acquisition of *in vivo* ^{1}H MR spectra has been difficult due to a number of methodological problems. First, water gives a signal at magnitude of 10^5 times greater than ^{1}H-detectable molecules found in tissue or lipid and thus water suppression techniques have been developed to lessen the magnitude of the water signal in ^{1}H spectra [Ernst and Hennig, 1995; van Vaals et al., 1991]. Second, large, mobility-restricted lipid molecules give broad signals in ^{1}H spectra that may interfere with signals of interest. Finally, due to the large number of molecules that can be detected using ^{1}H MRS, and the relatively small magnets that are used in human MRS, signal overlap occurs with a number of molecules, leading to poor resolution and sensitivity.

In vivo ^{1}H MRS is evolving and better acquisition sequences that optimize the signals of compounds of interest are being developed. Currently, *myo*-inositol, creatine+phosphocreatine (Cr+PCr), choline, and N-acetylaspartate (NAA) can be well resolved using *in vivo* ^{1}H MRS (Figure 2).

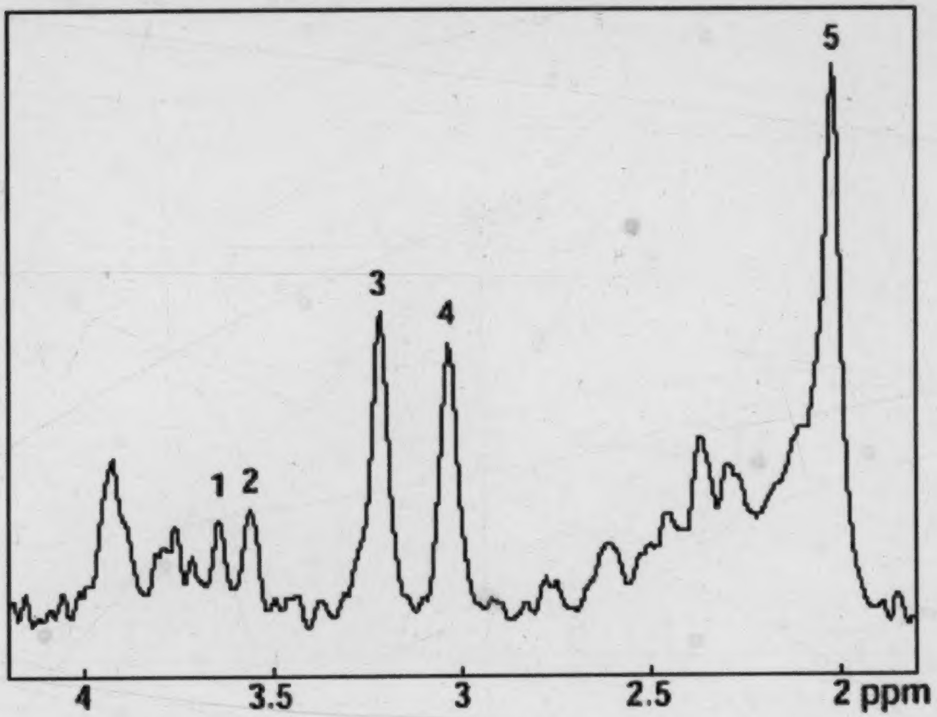

Figure 2: Human In Vivo ^1H MRS Spectrum of Temporal Lobe Brain Tissue. The peak assignments are as follows: 1 = myo-inositol 3.65 ppm; 2 = myo-inositol 3.56 ppm; 3 = choline; 4 = Cr+PCr; 5 = NAA

At magnet strengths greater that 2T, the *myo*-inositol multiplet peaks occur at chemical shifts of 3.56 and 3.65 ppm. However, the *myo*-inositol peak at 3.56 ppm co-resonates with signals from glycine and inositol monophosphates while the peak at 3.65 ppm co-resonates with signals from the inositol monophosphates [Cerdan et al., 1985; Behar and Ogino, 1991].

At magnetic strengths lower than 2T, the signals at 3.56 and 3.65ppm converge into a single broad peak.

Like *in vivo* ^{31}P MRS, the expression of data obtained from ^{1}H MRS have been inconsistent. Ratio values have been used by a number of researchers where the Cr+PCr or NAA signals have been used as the internal reference. Again, the assumption is that the signal selected to act as the denominator in ratio data is stable and unchanging in the presence of psychiatric illness, pharmaceutical agents, or environmental changes. The possible influence of these factors on Cr+PCr or NAA remains unresolved and, thus, caution must be exercised when interpreting ratio values from *in vivo* ^{1}H MRS. Recently, absolute quantification methods for *in vivo* ^{1}H MRS have been developed [Danielsen and Henriksen, 1994; Ala-Korpela et al., 1995], and in the future, these may resolve issues associated with the use of ratio values.

C. Effects of Lithium and Valproate on the PI-cycle

C.1. Introduction

While it is thought that lithium and valproate act by different mechanisms of action, similar effects on physiological systems may provide insights into the therapeutic mechanisms by which these drugs alleviate symptoms of Bipolar

Disorder. Lithium has a long history in the treatment of Bipolar Disorder and, thus, the body of literature covering lithium's effects on physiological systems is quite extensive. Due to its wide spectrum of physiological effects, uncovering a specific therapeutic mechanism of action for lithium has been difficult. In recent years, much of the research has focused on lithium's effects on second messenger systems and more specifically, the PI-cycle [Berridge et al., 1982]. In contrast, few studies have examined valproate's effects on the PI-cycle, and thus it is difficult to compare the two drugs in terms of similar and differential effects. However, the observed effects of both lithium and valproate on the PI-cycle will be reviewed in this chapter.

Valproate's mechanism of action in bipolar patients is generally associated with its effects on GABA and other amino acid neurotransmitters. The interaction of valproate and the GABA system has been extensively researched and valproate has been shown to have effects on GABA release, uptake, metabolism, and synthesis [Löscher, 1999]. In contrast, only a few studies, discussed later in the Introduction, have investigated lithium's effects on amino acid neurotransmitters. Thus, similarities and differences between the two drugs with regard to their effects on GABA and other amino acid neurotransmitters will be examined later in the chapter.

C.2. The PI-Cycle

The PI-cycle, outlined in Figure 3, is a second messenger pathway linked to serotonergic, adrenergic, cholinergic, and dopaminergic receptors [Nahorski et al., 1986; Fisher et al., 1992]. Agonist binding to any of these receptors in neural tissue causes a conformational change in the receptor. The receptor then binds to a membrane-associated, heterotrimeric G-protein comprised of α-, β-, and γ- subunits. A number of G-protein isoforms are known to exist, but the sub-family of Gq/11 proteins are commonly linked to the inositol phosphate signaling cascade [Exton, 1993]. The G-protein is activated when an agonist binds to a G-protein linked receptor. Activation of a given G-protein induces a conformational change in the α-subunit causing the dissociation of guanosine diphosphate (GDP) from the α-subunit and the replacement of GDP with guanosine triphosphate (GTP) [Exton, 1993]. The α-, and βγ-subunits then diffuse away from each other and interact with their respective targets while still attached to the cell membrane.

Phospholipase C (PLC) is targeted and activated by the α-subunit when PI-cycle linked receptors are stimulated by agonist binding. PLC hydrolyzes the bond between the phosphate group and the glycerol backbone of phosphatidylinositol 4,5-bisphosphate (PIP_2), generating the second messengers inositol 1,4,5-triphosphate ($Ins[1,4,5]P_3$), and *sn*-1,2-diacylglycerol (DAG) [Hughes and Putney, 1988]. $Ins[1,4,5]P_3$ is inactivated by the subsequent removal of phosphate groups by a family of phosphoinositol phosphatases to form a number of inositol bisphosphates and monophosphates, successively. The inositol monophosphates are then metabolized by IMPase to form *myo*-inositol. The inactivation of DAG occurs when it is metabolized to phosphatidic acid (PA), and then converted to cytidine diphosphate diacylglycerol (CDP-DG) [Berridge and Irvine, 1989]. Finally, *myo*-inositol and CDG-DG combine to form PI, and following the addition of two successive phosphate groups, PIP_2 is regenerated. Lithium has been found to inhibit IMPase [Hallcher and Sherman, 1980; Naccarato et al, 1974; Nahorski et al, 1991], resulting in a depletion of *myo*-inositol necessary for the regeneration of PIP_2. Thus, when agonist binding occurs at a PI-cycle linked receptor, it has been proposed that there will be less PIP_2 available for the generation of the second messengers, DAG and $Ins[1,4,5]P_3$.

In terms of their role as second messengers, Ins[1,4,5]P_3 diffuses into the cytosol and is responsible for the stimulation of Ca^{2+} release [Berridge, 1993], while DAG remains attached to the cell membrane and activates protein kinase C (PKC) isozymes [Huang, 1989]. Alterations at this level have been postulated to elicit short- and long-term changes in neurotransmitter release, protein phosphorylation, ion flux, gene transcription, and protein production [Berridge, 1994; Buchner, 1995], causing significant changes in the functioning of the cell. Therefore, pre-existing abnormalities in the PI-cycle may contribute to the abnormal behavioral components of Bipolar Disorder and thus, alteration of aberrant PI-cycle functioning by pharmaceutical agents may normalize behavior.

C.3. Lithium and the Inositol-Depletion Hypothesis

One of the more dominant hypotheses regarding lithium's mechanism of action is the "inositol-depletion hypothesis" first proposed by Berridge and colleagues [1982]. This followed initial observations that uncompetitive inhibition of IMPase by lithium [Hallcher and Sherman, 1980; Naccarato et al, 1974; Nahorski et al, 1991] leads to an accumulation of inositol monophosphates [Allison et al., 1976] and a corresponding depletion of *myo*-inositol [Allison and Stewart, 1971]. Lithium's proposed effects on the PI-cycle are outlined in Figure 3.

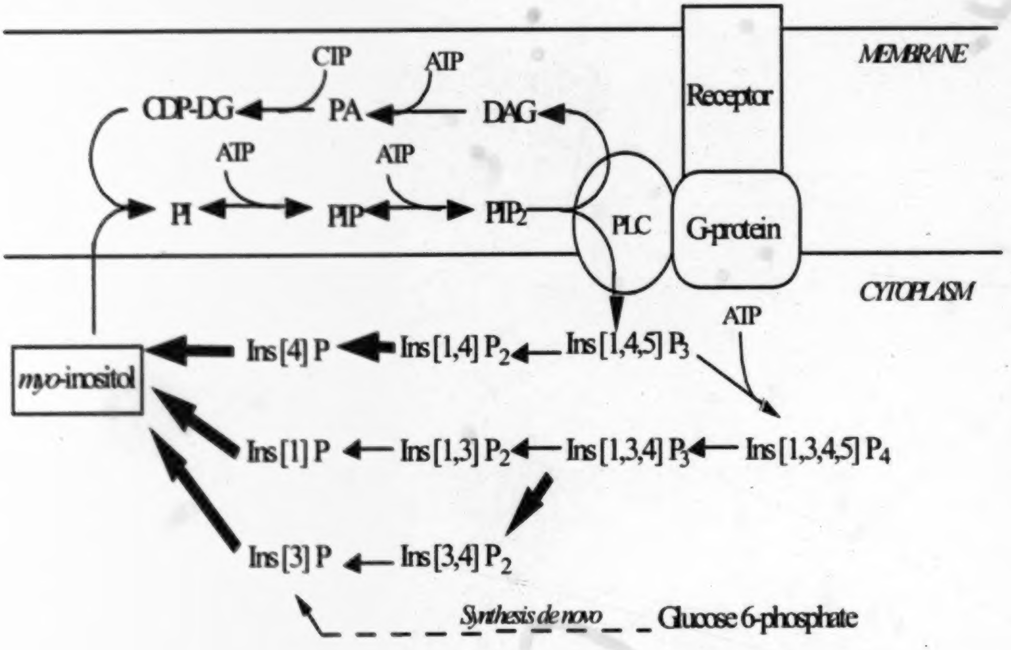

Figure 3: Phosphoinositol Cycle (PI cycle). Lithium blocks the enzyme, inositol monophosphatase, preventing the conversion of inositol-1-phosphate, (Ins[1]P), inositol-3-phosphate (Ins[3]P), and inositol-4-phosphate (Ins[4]P) to myo-inositol. It has been hypothesized that this leads to an accumulation of inositol monophosphates and a depletion of myo-inositol (steps blocked by lithium are shown by bold arrows). Reduced myo-inositol levels prevent the efficient formation of phosphatidylinositol (PI) from myo-inositol and cytidine diphosphodiacylglycerol (CDP-DG). Levels of phosphatidylinositol-4,5-bisphosphate (PIP$_2$), which is cleaved into two second messengers, inositol-1,4,5-triphosphate (Ins[1,4,5]P$_3$) and diacylglycerol (DAG) are subsequently decreased. An overall dampening of PI cycle functioning is thus postulated to occur. Of note, de novo synthesis of myo-inositol is also prevented in the presence of lithium as the metabolism of G6P to myo-inositol passes through Ins[3]P. Adapted from O'Donnell et al., 2000.

Since lithium inhibits IMPase, excessive activity in this enzyme has been implicated in the pathophysiology of Bipolar Disorder. However, when IMPase activity in red blood cells of manic drug free Bipolar patients was compared to that of healthy controls, no significant differences were observed [Moscovich et al., 1990]. In this same study, lithium was shown to inhibit the activity of IMPase by approximately 80%. Another study found that the activity of platelet IMPase isolated from lithium-treated manic patients was significantly lower than that of healthy controls [Moscovich et al., 1990]. Finally, IMPase activity in post-mortem samples obtained from bipolar patients has been found to be normal [Atack, 1996; Shimon et al., 1997]. These findings suggest that excessive IMPase activity is not part of the pathophysiology of Bipolar Disorder, but that the therapeutic mechanism of bipolar disorder may still be related to lithium's inhibition of IMPase via downstream effects on PI-linked second messengers resulting from a depletion of *myo*-inositol.

C.3.1. Evidence for the Inositol-Depletion Hypothesis in Laboratory Animals

While a number of researchers have examined the effects of lithium on levels of *myo*-inositol and inositol monophosphates in animals, few studies have administered chronic, therapeutic doses of lithium that would be representative of those used in patients receiving lithium treatment. The small number of *in vivo* studies that have administered therapeutic doses of lithium chronically have found a depletion of *myo*-inositol and an accumulation of inositol monophosphates in the cerebral cortex [Sherman et al., 1981; 1985], and various other regions [Hirvonen et al., 1991]. One study found a selective decrease in *myo*-inositol in the hypothalamus while finding no significant changes in other brain regions like the caudate, cerebellum, cortex, and hippocampus [Lubrich et al., 1997]. This study did not measure the effect of lithium on inositol monophosphates.

Acute administration of lithium to animals has also been carried out at therapeutic and at high doses that would generally be considered toxic in humans. In studies where high doses of lithium were acutely administered, the outcome was generally supportive of the inositol-depletion hypothesis in several brain regions [Hirvonen, 1991; Hirvonen and Savolainen, 1991; Hirvonen et al., 1988; Sherman et al., 1985; Preece et al., 1992]. However, an accumulation of inositol monophosphates but not a depletion of *myo*-inositol has been found at acute doses corresponding to low and high therapeutic drug levels in a number of brain regions [Hirvonen and

Savolainen, 1991; Savolainen et al., 1990]. Finally, in one study where low, intermediate, and high doses of lithium were administered acutely, no changes in *myo*-inositol were observed in the caudate, cerebellum, cortex, hippocampus, or hypothalamus [Lubrich et al. 1997]. However, following chronic administration of therapeutic doses, a depletion of *myo*-inositol in the hypothalamus was observed [Lubrich et al., 1997], indicating that acute treatment with therapeutic doses of lithium may not induce the same changes as long-term treatment.

C.3.2. Stimulation with Agonists and the Use of Radiolabelled Substrates

It has been suggested that lithium interferes with the production of second messengers derived from the PI-cycle in activated systems only [Berridge et al., 1982; 1989] since the recycling of *myo*-inositol from inositol phosphates is necessary for the resynthesis of PIP_2. Briefly, PIP_2 is metabolized to DAG and Ins $(1,4,5)P_3$ by PLC upon receptor stimulation of the PI-cycle (Figure 3). Many researchers have investigated the effects of receptor stimulation on the PI-cycle in lithium-treated rat brain. These studies normally involve *in vivo* administration of lithium followed by brain removal and preparation of cortical slices *ex vivo*. The brain slices are then incubated in a solution containing radiolabelled $[^3H]$*myo*-inositol in the presence or absence of an appropriate agonist. Following an incubation period, [^{3}H]*myo*-inositol and [^{3}H]inositol monophosphates are usually separated using anion exchange chromatography. The radioactivity of ^{3}H-labelled *myo*-inositol or inositol monophosphates is then counted by a liquid scintillation counter.

Using this methodology, very little support for the inositol-depletion hypothesis has been gathered and, as outlined in Table 1, the data have been wrought with inconsistencies. Interestingly, in cases where [^{3}H]*myo*-inositol has been administered to rodents by intracerebroventricular (i.c.v.) injection, followed by *in vivo* administration of a PI-cycle-linked receptor agonist before sacrifice, the inositol-depletion hypothesis has been supported [Sun et al., 1992; Whitworth et al., 1990].

Table 1: Radiolabelling/Agonist Stimulation Studies of the PI-Cycle.

Brain regions	Lithium		Agonist	Myo-inositol	IPs	Reference
	Treatment	Dose				
Cerebral cortex	Acute, ip 6-24 hours	10 meq/kg	basal	↓	↑	Sun et al., 1992
			mecamylamine	↓	↑	
			pilocarpine	↓	↑	
Cerebral cortex	Acute, ip 24 hours	10 meq/kg	5-HT	-	↓	Godfrey et al., 1989
			carbachol	-	N	
			noradrenaline	-	N	
Frontal cortex, striatum	Acute, ip 18 hours	6.75 meq/kg	basal	-	N	Li et al., 1993
			carbachol	-	N	
			noradrenaline		N	
Cerebral cortex, Hippocampus (mouse)	Acute, sc 18 hours	10 meq/kg	basal	-	↑	Whitworth et al., 1990
			pilocarpine	-	↑	
Cerebral cortex	Acute, sc 24 hours	3 meq/kg	pargyline	-	N	Honchar et al., 1990
			physostigmine	-	N	
			pilocarpine	-	N	
Frontal cortex, striatum	Sub-acute, ip 3 days	2.5 meq/kg/day	basal	-	↓(striatum)	Li et al., 1993
			carbachol	-	N	
			noradrenaline	-	N	
Cerebral cortex	Chronic, in food, 14 days	3 meq/kg b.i.d.	5-HT	-	↓	Elphick et al., 1988
			carbachol	-	↓	
			noradrenaline	-	↓	
Cerebral cortex	Chronic, in food, 30 days	1.7g/kg of food	basal	-	↓	Casebolt and Jope, 1987
			noradrenaline	-	↓	
Cerebral cortex, hippocampus (mouse)	Chronic, in food, 14 days	0.4% w/w of food	basal	-	N	Whitworth et al., 1990
			pilocarpine	-	↑	
Cerebral cortex, striatum (mouse)	Chronic, in food, 13 days	0.4% w/w of food	carbachol	↑ (cortex)	↓ (cortex)	Whitworth and Kendall, 1989
Cerebral cortex	Chronic, in food 28-52 days	40 meq/kg 28-52 days	pilocarpine	-	N	Honchar et al., 1990
			pargyline	-	N	
			physostigmine	-	N	
Frontal cortex, striatum	Chronic, ip 14 days	2.5 meq/kg /day	basal	-	↓(frontal cortex)	Li et al., 1993
			carbachol	-	N	
			noradrenaline	-	N.	
Frontal cortex, striatum	Chronic, ip 28 days	2.5 meq/kg /day	basal	-	↓(striatum)	Li et al., 1993
			carbachol	-	↓	
			noradrenaline	-	↓(frontal cortex)	
Cerebral cortex	Chronic, sc 3 or 16 days	3 meq/kg b.i.d.	5-HT	-	↓	Godfrey et al., 1989
			carbachol	-	↓	
			noradrenaline	-	↓	

While there are many inconsistencies in these data, several points need to be made regarding the radiolabelling of the PI-cycle. Isotopic equilibrium must be achieved in tissue labeled with a radioisotope if measurements are to be meaningful [Bradford and Rubin, 1986; Portilla and Morrison, 1986]. However, incubation times may vary with both the tissue and radiolabel used [Stubbs et al., 1988; Chandrasekhar et al., 1988, Horstman et al., 1988; Tarver at al., 1987; Portilla and Morrison, 1986; Bradford and Rubin, 1986; Koreh and Monoqo, 1986], sometimes requiring days to reach isotopic equilibrium. These long incubation times required with the use of *ex vivo* [^{3}H]*myo*-inositol may affect agonist response [Horstman et al., 1988]. However, the use of i.c.v. injections of [^{3}H]*myo*-inositol are likely to be more efficient than *ex vivo* incubations as *in vivo* incorporation of the radiolabel will occur more rapidly under the influence of functioning enzymes. Also, it has been demonstrated that inositol monophosphates and bisphosphates can be released from both phosphatidylinositol 4-monophosphate and phosphatidylinositol 4,5-bisphosphate [Singh, 1992; Brammer and Weaver, 1989]. Thus if pools of these two phospholipids are not labeled to isotopic equilibrium, the *ex vivo* radiolabelling method will provide inaccurate information regarding the metabolism of inositol monophosphates in tissues [Singh and Jiang, 1995]. Finally, lithium has been shown to alter G-protein functioning, which may have affected the outcome of radiolabelling experiments involving agonist stimulation of PI-cycle linked receptors (see below). With the above points considered, caution must be exercised when interpreting data obtained through radiolabelling of the PI-cycle.

C.3.3. Effects of Lithium on G-Proteins in Animals.

While a number of studies have examined the effects of mood stabilizers on the c-AMP pathway-linked G-proteins, G_s and G_i, [Colin et al., 1991; Lesch et al., 1991; Li et al., 1993; Jakobsen and Wiborg; 1998], only a few researchers have examined the effects of lithium on G_q and G_{11}, G-proteins specifically linking receptors to the PI-cycle. Since G_q and G_{11} act as a messengers between receptors and the PI-cycle, alterations in the activity or sensitivity of these G-proteins will affect the response of the PI-cycle following receptor stimulation. One study, examining the 5-HT-induced binding of [^{35}S]guanosine-5'-O(3-gamma-thio)triphosphate ([^{35}S]GTPγS) to $G\alpha_q$, found that [^{35}S]GTPγS binding was unchanged in rat cortex following one week of lithium administration but was reduced by 38% following six weeks of treatment [Wang and Friedman, 1999]. Another study examined phosphoinositide hydrolysis induced by the G-protein agonists, sodium fluoride (NaF) or GTPγS, and carbachol-induced potentiation of GTPγS. Phosphoinositide hydrolysis was found to be significantly attenuated in rat hippocampus, striatum, and cortex following four weeks of lithium administration but not following *in vitro* incubation of these regions with lithium [Song and Jope, 1992]. However, lithium has been found to have no effect on cortical G-protein levels, coupling, or activity following three weeks of administration [Li et al., 1993]. Nevertheless, there does appear to be some effect of lithium on G_q and G_{11} proteins following chronic administration in animals. Thus, it is possible that long-term lithium administration may alter the sensitivity of the PI-cycle upon receptor stimulation by acting on G-proteins. Chronic effects of lithium on G-proteins may also account for some of the anomalies observed in radiolabelling studies using agonist stimulation to study the inositol-depletion hypothesis.

C.4. Valproate and the PI-cycle

In contrast to lithium, valproate does not appear to inhibit IMPase [Vadnal and Parthasarathy, 1995]. In light of this observation, few studies have examined the effect of valproate on the PI-cycle as it has been assumed that valproate likely will not affect the levels of *myo*-inositol and inositol monophosphates in brain tissue. However, incubation of mouse brain slices with sodium valproate was shown to lead to an accumulation of Ins(1,4,5)P_3, but not inositol monophosphates [Dixon and Hokin, 1997]. Another study found a significant attenuation of striatal agonist-stimulated inositol phosphate formation following chronic sodium valproate treatment [Li et al., 1993]. Carbachol and norepinephrine were used as agonists in this study. These studies seem to suggest effects of valproate on the PI-cycle, but both used *ex vivo* radiolabelling in their experimental protocols. Thus, as suggested earlier, the use of *ex vivo* radiolabelling to study metabolism of inositol phosphates in the PI-cycle may lead to ambiguous results regarding inositol-depletion, and thus, caution must be exercised when interpreting these data.

C.5. Problems With Animal Data

Due to the wide range of doses, experimental protocols, and length and routes of administration used in animal studies investigating the effects of lithium on the PI-cycle, it is difficult to make generalizations about the data. Unfortunately many researchers have chosen to administer high, acute doses of lithium. Since these high doses (>5 meq/kg) are normally toxic in human systems, plasma and tissue levels of lithium may not reflect those found in patients receiving lithium treatment. Also, the use of acute versus chronic administration will only identify the short-term effects of drug treatment while possibly neglecting changes induced by the long-term treatment normally employed when treating Bipolar patients.

In high dose, acute lithium administration studies, the data are generally supportive of the inositol-depletion hypothesis [Allison et al., 1976; Hirvonen, 1991; Hirvonen and Savolainen, 1991; Sherman et al., 1985; Preece et al., 1992]. Using acutely administered, therapeutic doses of lithium, inositol monophosphate accumulation was observed in the absence of *myo*-inositol depletion in a number of brain regions [Savolainen, 1990; Hirvonen and Savolainen, 1991]. Studies that have examined *myo*-inositol and inositol monophosphates following chronic administration of therapeutic doses of lithium have been generally supportive of the inositol-depletion hypothesis [Sherman et al., 1981; 1985; Hirvonen et al., 1991; Lubrich et al., 1997]. One study found that a depletion of *myo*-inositol was only observed in the hypothalamus following chronic administration, but not following acute administration [Lubrich et al., 1997]. Thus the data suggest that both the dose and length of lithium administration may be responsible for differential effects on brain levels of *myo*-inositol and inositol monophosphates. However, the effects of lithium on the PI-cycle have not been correlated with the clinical efficacy of lithium in the treatment of Bipolar Disorder.

The differential effects of lithium on specific brain regions also need to be addressed in animal studies. The effects of lithium are proposed to be particularly evident in overactive cells and in cells whose inositol and lithium transport systems render them selectively vulnerable to the effects of lithium [Gani et al, 1993]. Berridge and colleagues [1989] initially proposed that lithium's effect on the PI-cycle was more substantial in the brain where the blood-brain barrier limited the transport of *myo*-inositol from the periphery into the brain, thus rendering brain neuronal cells particularly vulnerable to the effects of lithium. However, one study observed regional differences in uptake of *myo*-inositol into brain *in vivo* [Patishi et al., 1996]. Finally, while several studies have supported the inositol-depletion hypothesis in a number of brain regions, it has been recently observed that lithium causes a selective decrease in *myo*-inositol levels in the hypothalamus to the exclusion of other brain structures [Lubrich et al., 1997]. However, the length and dose of lithium administration vary among studies examining different brain regions, and, as suggested earlier, these variables may affect the levels of *myo*-inositol and inositol monophosphates *in vivo*. Thus the effects of lithium on regional brain levels of *myo*-inositol and inositol monophosphates need to be examined further.

C.6. Examination of the Inositol-Depletion Hypothesis in Human Subjects

C.6.1. Peripheral, CSF and Post-Mortem Measures of the Inositol-Depletion Hypothesis

Before the advent of MRS technology to study neurochemistry *in vivo*, researchers attempted to study lithium-induced changes in cerebrospinal fluid (CSF) and post-mortem brain tissue. One study, examining the effect of lithium administration at therapeutic doses on CSF *myo*-inositol levels in schizophrenics, found no significant changes following three days of treatment [Levine et al., 1993]. However, more than one pool of brain *myo*-inositol appears to exist [Bersudsky et al., 1994; Shayman and Wu; 1990] and thus CSF *myo*-inositol and brain *myo*-inositol may be equilibrating with different pools. Also, lithium does not lower plasma *myo*-inositol levels [Agam et al., 1995] and since CSF *myo*-inositol is derived mainly from plasma [Spector and Lorenzo, 1975], it is possible that CSF levels of *myo*-inositol are not reflective of those found in the brain. Therefore, the absence of changes in levels of CSF *myo*-inositol may not be reflective of lithium's effects on brain tissue and more specifically, on tissue from specific brain regions subject to differential uptake of lithium and *myo*-inositol.

Results from analysis of post-mortem brain tissue from bipolar patients have been supportive of lithium's effects on *myo*-inositol concentrations. Reduced *myo*-inositol levels were found in post-mortem brain tissue of euthymic, lithium-treated bipolar patients compared to those obtained from healthy controls [Belmaker et al., 1998]. These effects were specific to the frontal lobe and hypothalamus while no significant reductions in levels of *myo*-inositol were observed in the cerebellum or occipital lobe. Again, this evidence supports the idea that the effects of lithium may be regionally specific. One study found decreased formation of phosphoinositides in post-mortem tissue (occipital lobe) of medicated bipolar patients compared to healthy controls [Jope et al., 1996]. This study made use of agonist stimulation with either carbachol or 5-HT and the corresponding *ex vivo* incorporation of radioactive markers into the PI-cycle. Therefore this study may be subject to the same methodological problems as animal studies using *ex vivo* radioactivity incorporation methods.

Peripheral blood cell measures of the PI-cycle have also proven to be quite useful in the study of Bipolar Disorder. While the use of peripheral measures does have some limitations, the inositol-depletion hypothesis has been supported by research in peripheral blood cells. One group found that the activity of IMPase in platelets from lithium-treated manic patients was significantly lower that that of healthy controls [Moscovich et al; 1990]. This was supported by another study that observed an inhibition of IMPase activity following the incubation of erythrocytes obtained from healthy controls with therapeutic concentrations of lithium [Agam and Livne, 1989]. Finally, Greil and colleagues [1991] incubated neutrophils obtained from a group of male patients diagnosed with Bipolar, Major Affective or Schizoaffective Disorder and receiving lithium treatment, with formylmethionylleucylphenylalanine (fMLP), an agonist of the PI-cycle. Levels of [^{3}H]*myo*-inositol in patients were then compared to those in neutrophils from age- and sex-matched healthy controls and female patients. A significant attenuation of [^{3}H]*myo*-inositol formation was found in the male patients compared to the control groups.

C.6.2. ^{1}H MRS Measures of *Myo*-Inositol

The use of *in vivo* ^{1}H MRS to study brain levels of *myo*-inositol is a relatively new technique. Thus, very few studies have been published regarding the effects of mood stabilizers and psychiatric state on regional concentrations of brain *myo*-inositol. In the small number of studies that have been carried out, the method of quantification, brain region examined, and type of experimental subject have all varied.

Administration of lithium to manic bipolar patients for one week resulted in a reduction of *myo*-inositol from pre-treatment baseline concentrations in the frontal lobes [Yue et al., 1999]. Another study, examining depressed bipolar patients, found a reduction in *myo*-inositol levels from pre-treatment baseline levels in the right frontal lobe following 5-7 days and 3-4 weeks of lithium administration [Moore et al., 1999]. No changes were observed in the left temporal lobe, left parietal lobe, or the occipital lobe. Unfortunately, these studies did not include healthy controls in their experimental protocols and thus baseline levels of *myo*-inositol in manic and depressed bipolar patients could not be compared to those found in healthy controls. Such data would indicate whether these patients have abnormal levels of *myo*-inositol compared to healthy controls, further explaining the *in vivo* neurochemical effects of lithium on *myo*-inositol in bipolar patients. Therefore, the extent to which lithium decreases the *in vivo* concentration of *myo*-inositol in bipolar patients in a given mood state compared to healthy controls remains to be clarified.

In vivo levels of *myo*-inositol have also been examined in euthymic bipolar patients following chronic lithium treatment. No changes in the *in vivo* brain ratio of *myo*-inositol/(Cr+PCr) have been observed in the temporal lobe [Silverstone et al., unpublished data in this laboratory] or occipital lobe [Sharma et al., 1992] of lithium treated euthymic patients. These findings are supported by evidence that lithium does not affect *in vivo* brain levels of *myo*-inositol in the cerebral cortex of euthymic bipolar patients [Bruhn et al., 1993]. Also, lithium had no significant effects on the *in vivo* brain ratio of *myo*-inositol/(Cr+PCr) following lithium administration to healthy controls for one week [Silverstone et al., 1996; 1999]. Finally, a small study not consistent with others examining euthymic patients found an increase in the concentration of *myo*-inositol in the basal ganglia of three lithium-treated, euthymic patients compared to healthy controls [Sharma et al., 1992].

Overall, ^{1}H MRS studies indicate that there appears to be no difference in the concentration of *myo*-inositol in lithium-treated euthymic bipolar patients as compared to unmedicated healthy controls. Interestingly, these studies in lithium-treated euthymic patients may serve as evidence that lithium lowers or "normalizes" *in vivo* levels of *myo*-inositol in manic or depressed bipolar patients, causing the concentration of *myo*-inositol in euthymic patients to be comparable to that of healthy controls. This is supported by findings that lithium does not alter the *in vivo* concentration of *myo*-inositol in healthy controls [Silverstone et al., 1996; 1999]. However, lithium-induced reductions in *myo*-inositol concentrations *in vivo* have only been observed in the frontal lobes [Yue et al., 1999; Moore et al, 1999]. Studies involving euthymic patients have not investigated the effects of lithium on *myo*-inositol in the frontal lobes and have instead examined regions such as the occipital lobe, temporal lobe, cerebral cortex, and the basal ganglia. Thus, the regional effects of lithium on *in vivo* brain tissue of euthymic, manic, and depressed bipolar patients need to be clarified. Also, future ^{1}H MRS studies must investigate the relationship between bipolar psychiatric state and *in vivo* brain concentrations of *myo*-inositol in lithium-treated and untreated bipolar patients compared to healthy controls.

C.6.3. ^{31}P MRS Measures of Inositol Monophosphates

As mentioned previously, inositol monophosphates are one of several compounds co-resonating in the PME peak of ^{31}P spectra. Thus, any accumulation of inositol monophosphates that is predicted by the inositol-depletion hypothesis may be indirectly measured using *in vivo* ^{31}P MRS. A number of researchers have examined the PME peak in euthymic patients using *in vivo* ^{31}P MRS. In euthymic patients withdrawn from treatment for one week, decreased PME concentrations were observed in the frontal lobes [Deicken et al., 1995a] and temporal lobes [Deicken et al., 1995b] compared to healthy controls. Also, euthymic patients treated mainly with lithium had decreased PME concentrations in the frontal lobes compared to healthy controls [Kato et al., 1992; 1993; 1994a]. However, in euthymic bipolar patients treated with various medications, no change in the concentration of PME was observed in the frontal lobes [Kato et al., 1994a; 1995]. Finally, a decrease in the concentration of PME in lithium- and valproate-treated euthymic bipolar patients compared to healthy controls was found in the left temporal lobe [Silverstone et al., unpublished data]. This study made use of acute *d*-amphetamine as an *in vivo* model of mania [Jacobs and Silverstone, 1986], which is discussed later in this chapter.

Increased PME concentrations have been observed in the frontal lobes of medicated manic and depressed bipolar patients compared to healthy controls and euthymic patients [Kato et al., 1991; 1994b]. However, one study examining medicated manic bipolar patients found a decrease in the concentration of PME in the left frontal lobe [Kato et al., 1995]. The same study found no change in the concentration of PME in frontal lobes of medicated depressed patients. It is important to note, however, that the medications used in all four of the above studies were not controlled and thus patients were on a number of different medications. This inconsistency of drug treatment may be a confounding variable.

It is difficult to make generalizations about the *in vivo* ^{31}P MRS bipolar data in relation to the inositol-depletion hypothesis due to a number of methodological issues. First, studies focused on *unmedicated* euthymic bipolar patients were carried out following a one week period of drug withdrawal [Deicken et al., 1995a; 1995b]. Interestingly, the observed decreases in the PME concentrations of euthymic patients withdrawn from treatment are quite similar to the decreased PME concentrations observed in lithium- [Kato et al., 1992; 1993; 1994a; Silverstone et al., unpublished data] and valproate-treated [Silverstone et al., unpublished data] euthymic patients. Due to the long-term effects of drug treatment, changes observed in patients withdrawn from treatment may not be an adequate reflection of bipolar patients in an unmedicated, euthymic state. In essence, baseline abnormalities due to psychiatric state may be masked.

Also complicating the interpretation of the *in vivo* ^{31}P MRS data is the use of bipolar patients in different psychiatric states (i.e. depressed, euthymic, manic), and taking different medications. While increases in PME concentrations have been observed in both manic and depressed states of medicated bipolar patients [Kato et al., 1991; 1994b], there are no existing data with regard to unmedicated depressed and manic Bipolar patients. Thus, at present, the existence of trait- and state- dependent abnormalities in the PME concentrations of unmedicated bipolar patients has not been clarified. It is therefore difficult to assess the effects of drug treatment on *in vivo* PME concentrations of bipolar patients in different states by interpreting the existing data. Also, since different medications may have differential effects on *in vivo* PME concentrations, studies that include numerous medications in their protocol further complicate the interpretation of the data.

Silverstone and colleagues [1996] have attempted to clarify the effects of lithium on PME concentrations in the absence of psychiatric illness by administering lithium to healthy controls for a period of one week. This study found no changes in the concentrations of PME in the temporal lobe. In a separate group of healthy controls, lithium administration (one week) followed by acute *d*-amphetamine administration caused an increase in the concentration of PME in the temporal lobe of healthy controls [Silverstone et al., 1999]. While the administration of single drugs to groups of healthy controls seems like an ideal way to study drug mechanisms, this model neglects trait-dependent abnormalities that may exist in bipolar patients. Therefore, the effects of mood state on the PME peak in unmedicated bipolar patients must be clarified to identify the existence of possible state-dependent abnormalities in PME concentrations. In the presence of state- and trait-dependent abnormalities, drug treatment may elicit differential effects in PME concentrations of bipolar patients when compared to healthy controls.

C.6.4. Problems With *In Vivo* Human MRS Studies

Spectra obtained from human *in vivo* ^{1}H and ^{31}P MRS are both subject to poor resolution and sensitivity due to the low magnet strengths (1-4T) used in human subjects. One variable that may be interfering with the accurate assessment of changes in the inositol monophosphates may be the co-resonance of the inositol monophosphates with a number of other compounds. The inositol monophosphates contribute approximately 10% to the area of the PME peak [Silverstone et al., 1996] in ^{31}P MRS spectra, and thus changes in the inositol monophosphates must be of sufficient magnitude for statistically significant results to be observed. Also, the effects of psychiatric state and drug treatment on the concentrations of other large contributors to the PME peak (e.g. PC and PE) have gone unexamined and thus changes in this peak may not reflect changes in the inositol monophosphates.

Using ^{1}H MRS, *myo*-inositol, inositol monophosphates, and glycine all co-resonate in the peak (~3.6 ppm) commonly used for *in vivo* measurements of *myo*-inositol. Since the *in vivo* concentration of inositol monophosphates is postulated to increase in the presence of lithium, decreases in *myo*-inositol concentrations may be masked by increases in the concentration of inositol monophosphates. Additionally, changes in glycine concentrations may also confound *myo*-inositol data obtained using *in vivo* ^{1}H MRS [Jope et al., 1989; Löscher and Hörstermann, 1994; Martin-Gallard et al., 1985].

Quantitative *in vivo* ^{31}P MRS has not been used in the study of affective disorders, and researchers studying *in vivo* PME concentrations in bipolar patients and healthy controls have consistently used the ratio method of data expression. While absolute quantitative techniques have recently been developed for *in vivo* ^{1}H MRS, ratio data have also been used as a means of expressing results. As discussed earlier, ratio values involve the division of the area of a metabolite peak of interest by that of a compound or group of compounds thought to be stable *in vivo* under the influence of drug treatment or psychiatric state. Unfortunately, ratio methods of data expression have not been validated, and disease- and drug-induced changes in the concentrations of internal references may lead to inaccurate results. Consequently, changes in metabolites used as internal references due to medication or disease may in fact confound concentration changes in compounds under observation when these variables are presented as a ratio. Thus, the validity of ratio data obtained from *in vivo* ^{31}P MRS studies must be clarified.

Finally, more rigorous study designs will be needed in the future if MRS technology is going to be effective in studying the inositol-depletion hypothesis *in vivo*. Possible trait- and state-specific abnormalities in the *in* *vivo* concentrations of *myo*-inositol and PME must be identified in unmedicated bipolar patients before the effects of drug treatment will be fully understood. In the past, many researchers have compared patients receiving numerous medications to healthy controls to study trait-specific abnormalities in PME concentrations. These studies neglect the fact that drug treatment may also modify PME concentrations and that different drugs may have differential effects on PME concentrations. Also, trait abnormalities and drug effects may be specific to certain regions and thus, these regions must be identified in future studies.

In conclusion, *in vivo* MRS measurements have thus far been supportive of PI-cycle involvement in Bipolar Disorder in medicated and unmedicated patients. Unfortunately, patient heterogeneity and methodological issues have prevented the identification of conclusive explanations for findings obtained from MRS studies. However, further advances in MRS technology and the use of more controlled study designs should provide valuable information regarding possible PI-cycle abnormalities in Bipolar Disorder and the effects of different mood stabilizers on the PI-cycle in both patients and healthy controls. Finally, the inositol-depletion hypothesis can be examined further with regard to lithium and other mood stabilizers that may exert similar effects on the PI-cycle through related or unrelated mechanisms.

C.7. Summary and Implications of Research Related to the Inositol-Depletion Hypothesis

While the literature has been clouded with inconsistencies, the inositol-depletion hypothesis still remains an attractive explanation of lithium's therapeutic mechanism of action. The inositol-depletion hypothesis has gathered some support in animal studies, but a number of methodological issues have prevented the development of clear explanations for the effects of lithium in animals. Results from human studies are confusing and confounded by the inattention of researchers to issues such as heterogeneous use of medication, and potential pre-existing disease- and state-dependent abnormalities in the PI-cycle of bipolar patients before drug treatment. However, the collection of data from both humans and animals seems to support the existence of PI-cycle abnormalities in bipolar patients. Also supported is the alteration of the PI-cycle by lithium. Valproate also seems to have effects on the PI-cycle, but since very little research has been carried out in this area, it is too early to make generalizations about the interaction of valproate with the PI-cycle.

The efficient breakdown of inositol monophosphates to *myo*-inositol is crucial for the generation of the PI-cycle second messengers, DAG and Ins(1,4,5)P_3, which initiate a number of cellular reactions. Thus, abnormalities or changes induced by mood stabilizers have the potential to interfere with several cellular processes that may be related to behavior and behavior regulation. DAG and calcium are known activators of PKC isozymes and, thus, changes in the production of second messengers have the potential to alter patterns of protein phosphorylation by PKC enzymes.

A number of researchers have started to examine the effects of mood stabilizers such as lithium and valproate on PKC enzymes and gene transcription. Both drugs have been shown to have effects on PKC activity and transcriptional regulation, but since these data are beyond the scope of this thesis, interested readers are referred elsewhere [Manji et al., 2000]. Interestingly, researchers have not yet correlated these changes induced by lithium and valproate with mechanisms of action. It is possible that through down-regulation of the PI-cycle and its associated second messengers, lithium has indirect effects on PKC activity translating into long-term cellular changes. Thus, an understanding of the effects of lithium and other mood stabilizers on the PI-cycle is crucial for the development of hypotheses related to the effects of these drugs on enzymes or cellular processes initiated by activation of the PI-cycle.

D. Effects of Lithium and Valproate on Amino Acid Neurotransmitters

D.1. Introduction

GABA dysregulation has been proposed as a possible pathological abnormality in patients with mood disorders following the early observation that valproic acid, a GABA agonist, was effective in the treatment of Bipolar Disorder [Emrich et al., 1980]. Since then, the effects of valproate on GABA and other amino acid neurotransmitters have been extensively examined. Lithium's effects on amino acid neurotransmitters have received less attention, but several studies have reported effects of lithium on amino acid concentrations in animals. Thus, it may be useful to examine these data in the search for common mechanisms between lithium and valproate.

D.2. GABA

GABA is the most abundant inhibitory amino acid in the brain and is present in most areas of the brain. GABA is derived from glucose metabolism and more specifically, during the tricarboxylic acid cycle (TCA) when α-ketoglutarate is transaminated to glutamate by GABA α-oxoglutarate transaminase (GABA-T), followed by the conversion of glutamate to GABA by glutamic acid decarboxylase (GAD). While GABA-T is widely distributed in both neuronal and nonneuronal cells in the brain, the presence of GAD is restricted to cells that synthesize GABA. Once released into the synapse, GABA's actions are thought to terminate when re-uptake occurs by a high affinity GABA transporter [Clark and Amara, 1993]. GABA-T is responsible for degradation where GABA is converted to succinic semialdehyde. In a process called the GABA shunt, the removed amino group is then transferred to α-ketoglutarate, again forming glutamate.

A number of studies have found that GABA concentrations increase in specific brain regions following valproate administration [Simler et al., 1968; Iadarola and Gale, 1979; Löscher, 1982], with much of the increase occurring presynaptically [Löscher et al., 1985]. Increases of GABA concentrations have also been described in CSF [Löscher, 1982; Zimmer et al, 1980; Löscher and Siemes, 1984; 1985] and plasma [Löscher, 1982; Löscher and Schmidt, 1980; 1981] following sodium valproate administration. However, one study has found a decrease in plasma GABA following chronic valproate treatment of bipolar patients [Petty, 1996]. Further work in this area has found that valproate inhibits the GABA degrading enzyme, GABA-T [Godin et al., 1969; Maitre et al., 1978; Whittle and Turner, 1978; Löscher, 1980; Larsson et al., 1986; Löscher, 1981a; 1981b; 1993], while increasing the activity of GAD [Wikinski et al., 1996; Löscher 1981a; 1981b; Phillips and Fowler, 1982]. While it is clear that valproate has effects on the GABA system, the clinical and therapeutic significance of these findings needs to be clarified. Changes in the GABA system have not yet been correlated to an alleviation of clinical symptoms, and thus further research is needed.

In terms of lithium's effects on GABA concentrations in the brain, one study found no significant changes in the concentration of GABA in the cerebral cortex, hippocampus, striatum or substantia nigra following lithium treatment [Jope et al., 1989]. However, other studies have found lithium to increase the concentration of GABA in a number of brain regions following both acute and chronic administration [Marcus et al., 1986; Gottesfeld, 1976]. Thus the effects of lithium on the GABA system are not completely clear at present.

D.3. Glutamate

Glutamate is the most abundant excitatory amino acid in the brain. Like GABA, most glutamate molecules are derived from glucose metabolism where α-ketoglutarate, formed during the citric acid cycle, is transaminated by an aminotransferase to form glutamate [Peng et al., 1993]. Glutamate is also synthesized from glutamine by the ATP-requiring mitochondrial enzyme, glutaminase. Following presynaptic release, the synaptic actions of glutamate are terminated when it is removed from the synapse by high affinity transporters.

At present the effects of valproate on glutamate are not clear. No effects of valproate on regional brain glutamate concentrations have been found in brain regions by several researchers [Löscher and Hörstermann, 1994; Biggs et al., 1992; Rowley et al., 1995]. However, one study found increased glutamate concentrations in cerebral cortex slices following incubation with valproate [Dixon and Hokin, 1997]. Therefore, more research is required to elucidate the effects of valproate on brain glutamate concentrations.

Lithium has been reported to increase the concentration of glutamate in several brain regions. Increases in the concentration of glutamate following chronic lithium treatment have been observed in the amygdala [Gottesfeld, 1976], brain stem [Marcus et al., 1986], cerebral cortex [Marcus et al., 1986], and hypothalamus [Gottesfeld, 1976]. It has been observed that lithium causes an increase in extracellular glutamate in a time- and concentration-dependent manner [Dixon and Hokin, 1996] while inhibiting the re-uptake of glutamate after acute administration [Dixon and Hokin, 1998]. Therefore the effect of lithium on the glutamate system may vary in a region- and time-dependent manner. However, decreases in brain glutamate concentrations have been observed in whole brain [Plenge, 1976], and the substantia nigra following lithium administration [Jope et al., 1989].

D.4. Other Amino Acid Neurotransmitters

Valproate has been found to increase glycine concentrations in rat brain following chronic administration [Martin-Gallard et al., 1985]. However, decreases in glycine concentrations have been observed in the pons and medulla of rat brain following chronic valproate treatment [Löscher and Hörstermann, 1994]. Lithium has been found to have no effects on brain glycine levels in chronically treated rats [Jope et al., 1989].

Reductions in the concentration of the brain excitatory amino acid aspartate have been observed following administration of both valproate [Chapman et al., 1982; Löscher and Hörstermann, 1994] and lithium [Jope et al., 1989]. However, lithium has also been found to increase the concentration of aspartate in the brain stem following both chronic and acute administration [Marcus et al., 1986].

A study examining the effects of lithium on regional brain taurine showed no significant changes in the levels of this amino acid in cerebral cortex, hippocampus, striatum, or substantia nigra following chronic treatment [Jope et al., 1989]. Valproate however, appears to increase the concentration of taurine in whole brain [Patsalos and Lascelles, 1981] and, more specifically, in the hippocampus [Löscher and Hörstermann, 1994].

Finally, lithium has been shown to increase the concentration of alanine in the substantia nigra following chronic administration while no changes were observed in the cerebral cortex, hippocampus, or striatum [Jope et al., 1989]. The effects of valproate on alanine concentrations have not been studied.

D.5. Summary and Analysis of Amino Acid Studies

Due to the range of inconsistencies observed in amino acid studies with lithium and valproate, it is difficult to make generalizations about the currently available data. However, it appears that lithium and valproate may have common effects on GABA and aspartate, while acting differentially on glutamate and taurine.

One of the methodological issues that makes many of these results difficult to compare is the fact that a number of different brain regions were examined by different researchers and, in some cases, whole brain was assayed. The effects of drug treatment may differ between brain regions and, in some cases, net changes may occur in whole brain that are different from concentration changes observed in smaller regions of the brain. Since regional effects are likely to be observed with each individual drug, it is difficult to compare the effects of lithium and valproate when each drug may affect specific brain regions differently. Therefore, the regional effects of lithium and valproate on amino acid neurotransmitters must be elucidated before clear comparisons can be made between the two drugs.

Finally, while lithium and valproate appear to have effects on brain concentrations of amino acids, it is still unclear whether the observed changes can be correlated to therapeutic mechanisms of action. Therefore, the relationship between the alleviation of bipolar symptoms and changes in one or several amino acids must be examined. Also, advances in ^{1}H MRS may soon allow researchers to routinely examine amino acid concentrations *in vivo*. This will enable researchers to identify possible disease- and trait-specific abnormalities in the amino acid concentrations of bipolar patients in addition to being able to study the effects of specific drugs in healthy and affected brain tissue.

E. *d*-Amphetamine Administration as a Model of Mania

It has been hypothesized that administration of *d*-amphetamine may serve as a useful model of mania [Jacobs and Silverstone, 1986]. In contrast, there is evidence that lithium does not attenuate the behavioral effects of *d*-amphetamine in animals [Fessler et al., 1982; Cappeliez and Moore, 1990] or healthy volunteers [Silverstone et al., 1998]. However, magnesium valproate has been shown to counteract locomotor hyperactivity caused by a mixture of *d*-amphetamine and chlordiazepoxide [Cao and Peng, 1993].

Acute *d*-amphetamine administration has been tested as a possible *in vivo* stimulant of the PI-cycle [Silverstone et al., 1999]. *d*-Amphetamine has been shown to increase extracellular concentrations of dopamine, noradrenaline, serotonin, and glutamate [Hoebel et al, 1989; Seiden et al., 1993; Karler et al., 1994; Reid et al., 1997; Kuczenski and Segal, 1997]. These neurotransmitters can then stimulate their respective receptors, causing an activation of the PI-cycle. An *in vivo* MRS study of lithium-treated healthy controls found that acute *d*-amphetamine administration led to significant changes in the concentrations of *myo*-inositol and PME that were not present in baseline scans [Silverstone et al., 1999]. Thus, the use of *d*-amphetamine as an *in vivo* stimulant of the PI-cycle appears promising, but further research is required to elucidate the effects of acute *d*-amphetamine on the PI-cycle.

F. Hypotheses and Aim of Study

F.1. Part I: The Use of NMR/MR Spectroscopy to Study the Inositol-Depletion Hypothesis in Rat Brain Extracts and in Human Subjects

The primary hypotheses of this study were threefold:

(1) In accordance with the inositol-depletion hypothesis, therapeutic doses of lithium will decrease the concentration of *myo*-inositol and increase the concentration of inositol monophosphates in whole brain of chronically treated rats.

(2) Therapeutic doses of valproate will not affect the concentrations of *myo*-inositol and inositol monophosphates in whole brain of chronically treated rats.

(3) Acute *in vivo* *d*-amphetamine administration will stimulate the PI-cycle, causing observed effects of lithium or valproate on concentrations of *myo*-inositol and the inositol monophosphates to be potentiated.

The aim of this study was to examine the neurochemical effects of lithium and valproate on the absolute concentrations of compounds that are of primary importance to researchers by using *in vivo* MRS to study the inositol-depletion hypothesis. This was accomplished using high-resolution (11.8T) NMR spectroscopy.

F.2. Part II: The use of NMR spectroscopy to study the effects of lithium and valproate on amino acid concentrations in rat brain

The primary hypotheses of this study were as follows:

(1) NMR spectroscopy will be a useful quantitative tool for studying the concentrations of amino acids in brain extracts.

(2) Lithium and valproate will have differential effects on brain concentrations of amino acid neurotransmitters.

The aim of this study was to compare the quantitative effectiveness of high-resolution (11.8T) NMR spectroscopy in measuring amino acids to a more established tool in neurochemical research, HPLC. This was accomplished by examining, with either high-resolution NMR spectroscopy or HPLC, the concentration changes of several amino acids following chronic lithium, valproate or saline treatment.

Materials and Methods

A. Methods 1: Measurement of Brain Neurochemicals in Whole Brain Extracts of Rats Chronically Treated with Lithium or Sodium Valproate Using NMR Spectroscopy

A.1. Chemicals

Table 2: Chemicals Used in Method 1

Chemicals	**Supplier**
Chloroform	Fisher Scientific (Fair Lawn, NJ, USA)
d-Amphetamine	SmithKline, &French (Philadelphia, PA, USA)
Deuterated water (D_20)	Aldrich Chemical Co. (Milwaukee, WI, USA)
Isopentane	Fisher
Lithium chloride	Fisher
Methanol (MeOH)	Fisher
Methylenediphosphonic acid trisodium salt tetrahydrate (DSS)	Sigma Chemical Co. (St. Louis, MO, USA)
Saline	Fisher
Sodium 3-trimethylsilyl [2,2,3,3, -^{2}H] propionate (TSP)	Canadian Isotopes (Pointe-Claire, Que.)
Sodium valproate	Sigma

A.2. Drug Administration and Sacrifice of Animals

This study was approved by the Health Sciences Animal Welfare Committee and all procedures were carried out in accordance with the guidelines of the Canadian Council on Animal Care. Adult male Sprague-Dawley rats (Ellerslie Biosciences, weighing 250-350 g were housed in plexiglass cages. The rats were given free access to food and water and were maintained on a 12-hour light/12-hour dark cycle. Injections of lithium, sodium valproate, and saline were started four days after the rats arrived, giving them an opportunity to adjust to their new environment.

Each rat received twice daily (b.i.d) intraperitoneal (IP) injections of either 2.0 mmol/kg lithium chloride [n=9], 300 mg/kg sodium valproate [n=9], or saline [n=9] at 07.30h and 16.00h for 14 days. Sodium valproate was initially administered in a dose of 400 mg/kg b.i.d, but this was decreased after 2 days when the rats exhibited symptoms of excessive sedation. All injections were administered in volumes of 2 ml/kg, and the doses used in this study have been previously shown to produce therapeutic serum levels of lithium [Ghoshdastidar et al., 1989] and valproate [Chen et al., 1999] in rats.

On day 15, the rats were administered their morning injection followed by an acute injection of 3.0 mg/kg of *d*-amphetamine or saline after 120 min. Thirty minutes later, they were decapitated. The brains were rapidly removed, immediately immersed in ice-cold isopentane, transferred to another set of vials, and then maintained at -80°C until extract preparation and NMR analysis.

A.3. Preparation of Extracts for NMR Analysis

Samples were prepared using a modified version of the total lipid extraction method as described by Bligh and Dyer [1959]. Whole rat brains were homogenized in 4 volumes of MeOH/chloroform (2:1,v/v). This was followed by the subsequent additions of one part of chloroform with homogenization, and one part of water with homogenization. Ten mls of homogenate were transferred to a test tube and centrifuged at 1,000 g for 15 min in a bench top centrifuge (Sorvall GLC-2B, Dupont, Wilmington, DE, USA). Following centrifugation, 3.5 ml of the water/MeOH layer was transferred to a 100 mm x 13 mm screw cap culture tube and maintained at -20°C overnight. The next day, samples were taken to dryness using vacuum centrifugation (Speed Vac, Savant) and then reconstituted in 0.83 ml of deuterated water (D_20) containing the ^{1}H NMR standard, sodium 3-

trimethylsilyl [2,2,3,3, -^{2}H] propionate (TSP) and the ^{31}P NMR standard, methylenediphosphonic acid trisodium salt tetrahydrate (DSS), at concentrations of 1.56 mM and 0.98 mM respectively. In addition to acting as internal chemical shift references, these two compounds made quantification of metabolite concentrations possible.

A.4. Acquisition of ^{31}P and ^{1}H NMR Spectra

^{31}P NMR spectra of the extracts were recorded at 11.75 T on a Varian Unity 500 NMR spectrometer using 45 degree (10 μs) pulses and an acquisition time of 1.6 s spanning 20kHz. Typical spectra were acquired for 3-4000 scans with ^{1}H broad band decoupling using a standard Varian broad band 5 mm NMR probe. ^{1}H NMR spectra of the extracts were obtained on the same spectrometer using 40 degree (4 μs) pulses, 32 scans/spectrum, and an acquisition time of 3 s spanning 8 kHz.

A.5. Spectral and Statistical Analysis

Spectral analysis was carried out blind to the drug treatment received by the animals. After noise filtering and baseline correction, peak areas in extract spectra were calculated using a Gaussian total line shape analysis using the Peak Research (PERCH) spectrum analysis software package (distributed by

PERCH project, Department of Chemistry, University of Kuopio, Kuopio, Finland). Concentrations were determined for alanine, aspartate, creatine+phosphocreatine (Cr+PCr), GABA, glutamate, glycine, *myo*-inositol, N-acetylaspartate (NAA), and taurine using ^{1}H NMR while concentrations for inositol monophosphates+phosphoethanolamine (IP_1+PE), G6P, PC, glycerophosphoethanolamine (GPE), and glycerophosphocholine (GPC) were determined using ^{31}P NMR. All metabolite concentrations were calculated by comparing peak areas to that of the added internal chemical shift standards of known concentration in ^{1}H and ^{31}P spectra respectively. Calculations are detailed in Appendix 1.

To determine significant changes in concentration, two-way analysis of variance (ANOVA) with Tukey *post-hoc* analysis was employed (SPSS for Windows, Release 7.5.1) with chronic drug (saline, lithium, or sodium valproate) and acute drug (saline, *d*-amphetamine) as the two factors. Results were deemed significant at $p \leq 0.05$.

B. Methods 2: Measurements of Amino Acid Neurotransmitter Concentrations Using HPLC

B.1: Chemicals

Table 3: Chemicals Used in Method 2.

Chemicals	**Supplier**
Acetonitrile	Fisher Scientific (Fair Lawn, NJ, USA)
Alanine	Sigma Chemical Co. (St. Louis, MO, USA)
Aspartate	Sigma
Fluoraldehyde Reagent	Pierce Chemicals (Rockford, Ill, USA)
GABA	Sigma
Glutamate	Sigma
Glycine	Sigma
Isopentane	Fisher
Lithium chloride	Fisher
MeOH	Fisher
Sodium hydroxide (NaOH)	Fisher
NaH_2PO_4	Fisher
Sodium valproate	Sigma
Taurine	Sigma
Tetrahydrofuran (THF)	Fisher

B.2. Drug Administration and Sacrifice of Animals

This study was approved by the Health Sciences Animal Welfare Committee and all procedures were carried out in accordance with the guidelines of the Canadian Council on Animal Care. Adult male Sprague-Dawley rats

(Ellerslie Biosciences) weighing 250-350 g were housed in plexiglass cages. The rats were given free access to food and water and were maintained on a 12-hour light/12-hour dark cycle. Injections of lithium, sodium valproate, and saline were started 4 days after the rats arrived, giving them an opportunity to adjust to their new environment.

Each rat received twice daily (b.i.d) intraperitoneal (IP) injections of either 2.0 mmol/kg lithium chloride [n=10], 300 mg/kg sodium valproate [n=10], or saline [n=10] at 07.30h and 16.00h for 14 days. All injections were administered in volumes of 2 ml/kg and the doses used in this study have been previously shown to produce therapeutic serum levels of lithium [Ghoshdastidar et al., 1989] and valproate [Chen et al., 1999] in rats.

On day 15, the rats were administered their morning injection and 150 min later they were decapitated. The brains were rapidly removed, immediately immersed in ice-cold isopentane, transferred to another set of vials, and then maintained at -80°C until extract preparation and HPLC analysis.

B.3. Preparation of Brains for HPLC Analysis of Amino Acids

Homogenization of brains and the analysis of amino acid concentrations was carried out using a modification of the procedure of Sloley et al. [1992]. In the following procedure, all mixtures were maintained on ice throughout the preparation process. Brains were homogenized in 5 volumes of ice-cold distilled water. Within 10 min of homogenization, 100 μl of homogenate were added to 400 μl of ice-cold MeOH. This mixture was then re-homogenized and allowed to sit on ice for 10 min followed by centrifugation in a microfuge for 2 min. The supernatant was then transferred to a microfuge tube and maintained at -80°C until the day of analysis. On the day of analysis, 50 μl of supernatant were added to 450 μl of ice cold water. This mixture was then centrifuged and the supernatant was transferred to HPLC vials.

B.4. HPLC Analysis

B.4.1. OPA Reagent.

This method involves determination of the levels of the amino acids using fluorometric detection after formation of o-phthalaldehyde (OPT) derivatives and has been previously described by Parent et al. [2000]. In an alkaline medium with 2-mercaptoethanol as a reducing agent, OPT (obtained as Fluoraldehyde reagent from Pierce Chemicals) reacts with primary amines to form highly fluorescent thioalkyl substituted isoindoles. Fluoraldehyde reagent is highly sensitive to oxygen and thus it was stored under N_2 and maintained at 4°C. In this experiment, a 5 µl portion of sample, standard, or blank was taken up and added to 5 µl of Fluoraldehyde reagent. For 1.5 min before injection, the sample/Fluoraldehyde mixture was held in the injection loop of an Alliance 2690XE solvent management system (Waters).

B.4.2. HPLC Set-up.

Mobile Phase "A": 900 ml 0.08 M Na_2PO_4 (8.8g), 240ml MeOH, and 20 ml acetonitrile were mixed together. NaOH (10N) was used to adjust the solution to pH 6.2. Following pH adjustment, 10 ml of THF was added to the solution, which was then filtered and degassed under vacuum, using 0.2 µm pore filters.

Mobile Phase "B": 1340 ml 0.08 M Na_2PO_4 (6.566g), and 1110 ml MeOH were mixed together. NaOH (10N) was used to adjust the solution to pH 6.2. Following pH adjustment, 60 ml of THF was added to the solution, which was then filtered and degassed under vacuum, using 0.2 µm pore filters.

Gradient: A mixture of 70% A: 30% B was used for 10 min at a flow rate of 0.5ml/min. The gradient was changed to 100% B for 10 min. followed by an increase in flow rate to 0.7 ml/min at 12 min. At 20 min, the flow rate was increased to 1.0 ml/min, and at 40 min a return to the initial conditions occurred. These conditions were maintained for 20 min to condition the column for the next run.

A Waters µBondapak C18 precolumn and a Waters spherisorb ODS2, C18 column (4.6x250 mm, 5 µm) were maintained at a constant temperature of 30°C. A Water Alliance 2690 XE pump and sample management system was coupled with a Waters 474 fluorescence detector with a 12 µl quartz flow cell; excitation and emission wavelengths of 260 nm and 455 nm, respectively were used. All samples, standards, and blanks were held at 4°C.

The amino acids eluted in the following order: aspartate, glutamate, asparagine, serine, glutamine, glycine, taurine, alanine, and GABA (Figure 4).

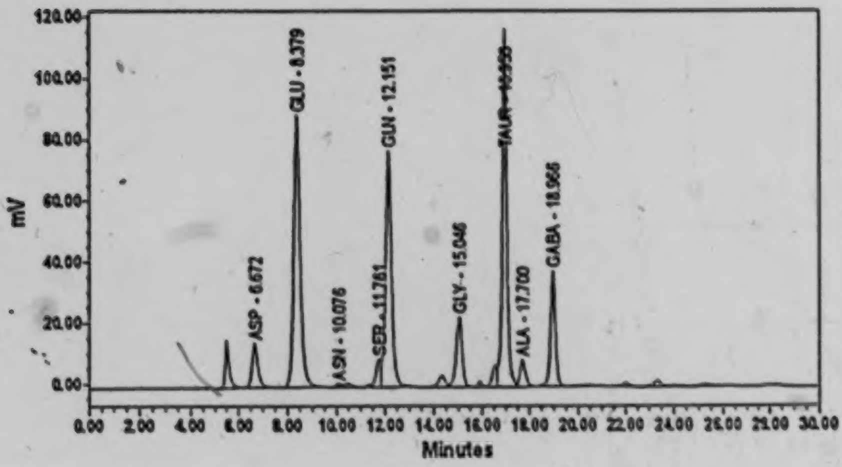

Figure 4: HPLC Chromatograph of Amino Acids Isolated from a Control Rat Brain. ASP=aspartate, GLU=glutamate, ASN=asparagine, SER=serine, GLN=glutamine, GLY=glycine, TAUR=taurine, ALA=alanine

B.4.3. Standard Solutions and Determination of Amino Acid Concentrations.

All standards were prepared in a 20% MeOH solution. The concentrations of amino acids in each standard solution are illustrated in Table 3 and calibration plots for each amino acid are illustrated in Appendix II. Calibration plots were used to determined the amount of each individual amino acid present in samples. To determine significant changes in amino acid concentrations, one-way analysis of variance (ANOVA) with Dunnett's t-test *post-hoc* analysis was employed (SPSS for Windows, Release 7.5.1). Results were deemed significant at $p \leq 0.05$.

Table 4: Concentrations of Amino Acids in Standard Solutions

Amino Acid	Standard 1 (µg/ml)	Standard 2 (µg/ml)	Standard 3 (µg/ml)	Standard 4 (µg/ml)	Standard 5 (µg/ml)	Standard 6 (µg/ml)
Aspartate	0	0.1	0.25	0.5	1.0	2.5
Glutamate	0	0.4	1.0	2.0	4.0	10.0
Glycine	0	0.05	0.125	0.25	0.5	1.25
Taurine	0	0.125	0.25	0.5	1.0	2.5
Alanine	0	0.05	0.125	0.25	0.5	1.25
GABA	0	0.2	0.5	1.0	2.0	5.0

Results

A. Study 1: The Effects of Lithium and Valproate on the Concentrations of *Myo*-inositol and the Inositol Monophosphates Using High-resolution NMR Spectroscopy

A.1. ^{1}H NMR Spectroscopy

Myo-inositol has been previously shown to give multiplet signals at 3.28, 3.54, 3.62, and 4.06 ppm in ^{1}H NMR spectra of rat brain extracts [Behar and Ogino, 1991]. Because the signals at 3.54 and 3.62 ppm are well resolved and relatively free of overlap from other metabolite signals, an average area of these two multiplets was used to quantify *myo*-inositol concentrations in the brain extracts. Figure 5 shows a cropped 500 MHz ^{1}H NMR spectrum of the brain extracts focussed on the region where these two peaks are located. Concentrations for NAA, glycine, and creatine were calculated using the singlet peaks at approximately 2.02, 3.56, and 3.93 ppm respectively [Behar and Ogino, 1991].

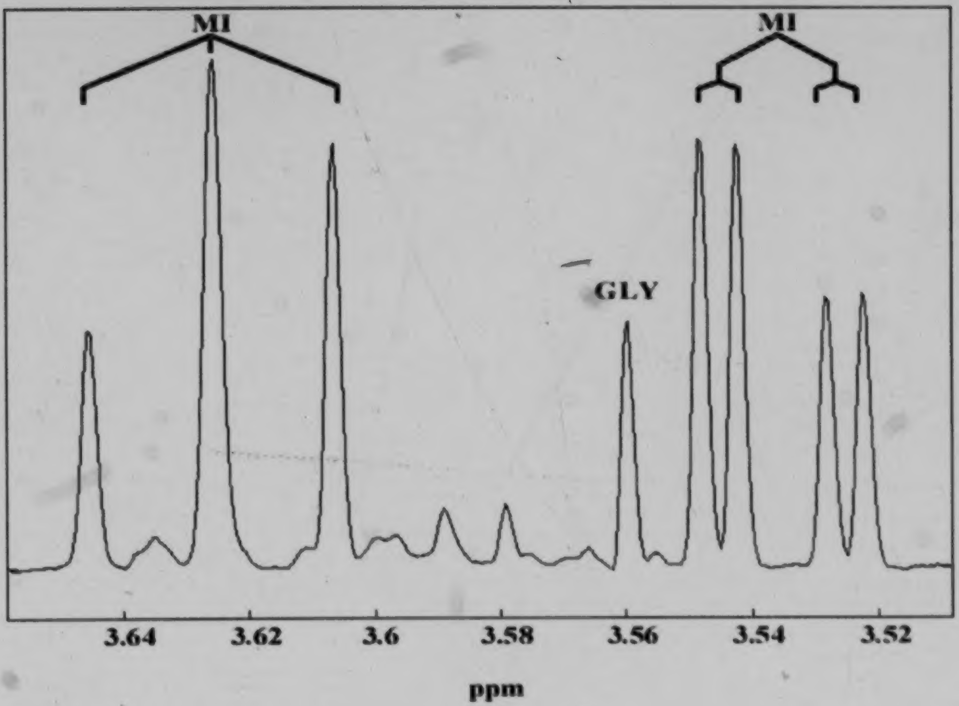

Figure 5: 500Mz ^{1}H NMR Spectrum of Rat Brain Extract Between 3.51 and 3.66 ppm. MI=myo-inositol; GLY=glycine. Adapted from O'Donnell et al., 2000.

Whole brain concentrations (μmol/g wet weight) of *myo*-inositol after chronic lithium, sodium valproate, or saline administration are illustrated graphically in Figure 6 and numerically in Table 5.

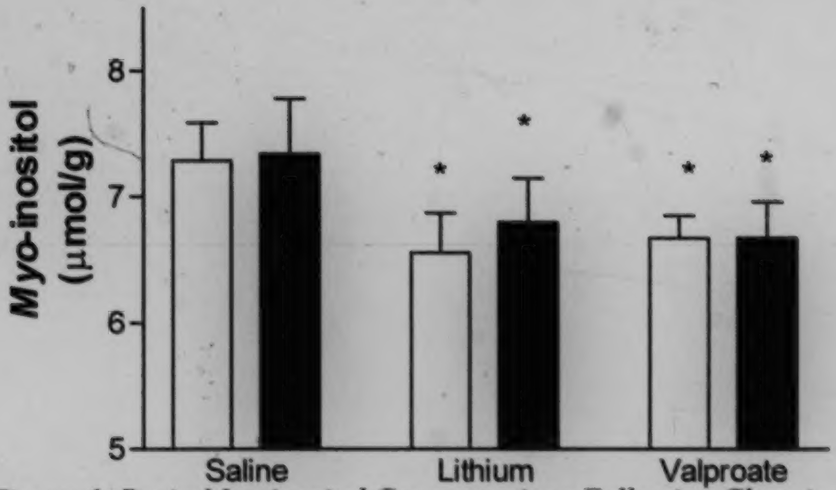

Figure 6: Brain Myo-inositol Concentrations Following Chronic Lithium and Valproate Treatment and Acute d-Amphetamine Administration to Rats. Values represent mean±SEM [n=9]. ■ *= d-amphetamine;* ☐ *= no d-amphetamine; * = statistically significant versus saline-saline-treated animals. Adapted from O'Donnell et al., 2000.*

Significant drug effects of lithium and sodium valproate were observed (F=23.559, df=2, p<0.001). Tukey *post-hoc* results showed that both lithium- (p<0.001) and valproate-treated (p<0.001) rats exhibited significantly decreased whole brain *myo*-inositol concentrations compared to saline-treated rats. *d*-Amphetamine had no effect on *myo*-inositol concentrations (F=1.274, df=1, p<0.27).

As shown in Figures 7 and 8, significant drug effects of lithium and sodium valproate were also observed with Cr+PCr (F=16.284, df=2, p<0.001) and

NAA (F=24.061, df=2, $p<0.001$). Tukey *post-hoc* results showed that both lithium ($p<0.001$) and valproate ($p<0.001$) treated rats exhibited significantly decreased whole brain creatine and NAA concentrations compared to saline-treated rats. Whole brain concentrations of Cr+PCr and NAA following chronic saline or drug administration are illustrated in Table 5. *d*-Amphetamine had no effect on Cr+PCr (F=0.100, df=1, $p<0.76$) or NAA (F=0.206, df=1, $p<0.66$) concentrations.

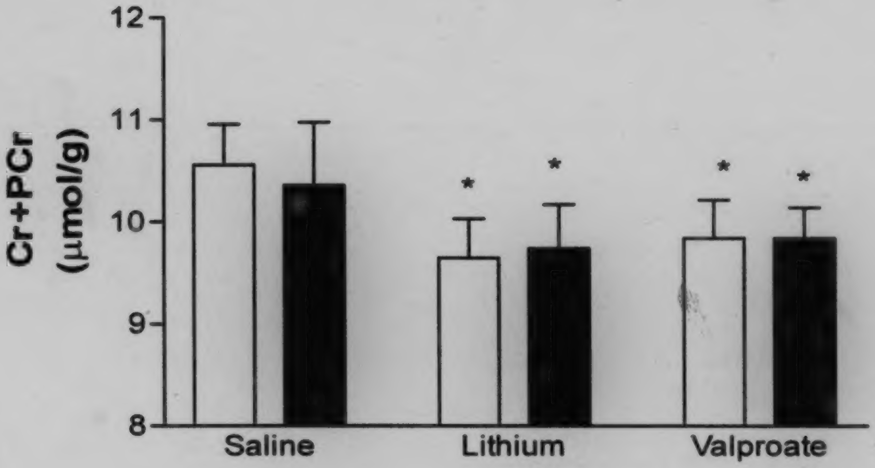

*Figure 7: Brain Cr+PCr Concentrations Following Chronic Lithium and Valproate Treatment and Acute d-Amphetamine Administration to Rats. Values represent mean±SEM [n=9]. ■ = d-amphetamine; □ = no d-amphetamine; * = statistically significant versus saline-saline-treated animals. Adapted from O'Donnell et al., 2000.*

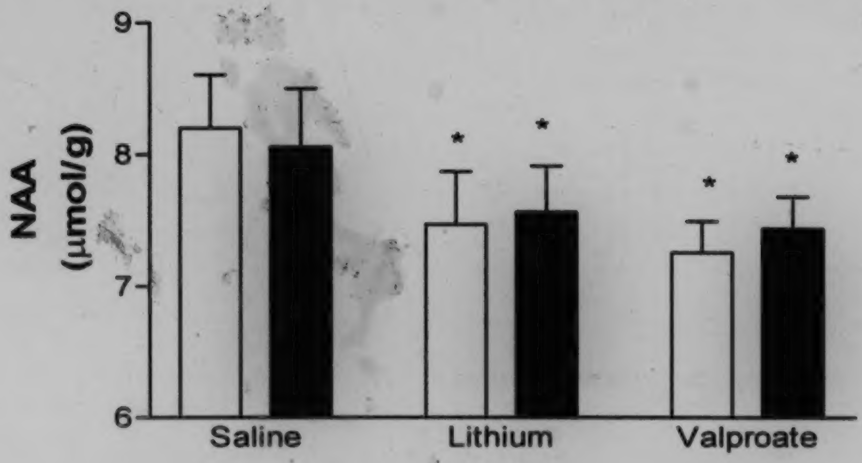

Figure 8: Brain NAA Concentrations Following Chronic Lithium and Valproate Treatment and Acute d-Amphetamine Administration to Rats. Values represent mean±SEM [n=9]. ■ *= d-amphetamine;* □ *= no d-amphetamine; * = statistically significant versus saline-saline-treated animals. Adapted from O'Donnell et al., 2000.*

No significant drug effects were observed with whole brain concentrations of glycine (F=2.607, df=2, p<0.09). Whole brain concentrations of glycine following chronic saline or drug administration are illustrated numerically in Table 5. *d*-Amphetamine had no effect on whole brain glycine concentrations (F=2.110, df=1, p<0.16).

A.2. ^{31}P NMR Spectroscopy

Figure 9 shows a typical 202.3MHz ^{31}P NMR spectrum of brain extract. It should be noted that the inositol monophosphates (IP_1) are co-resonant with phosphoethanolamine (PE) in the region around 6.4 ppm [Preece et al., 1992]. Because it is not possible to resolve the IP_1 signal from the PE signal, a combined concentration for these two compounds was calculated from the observed peak area.

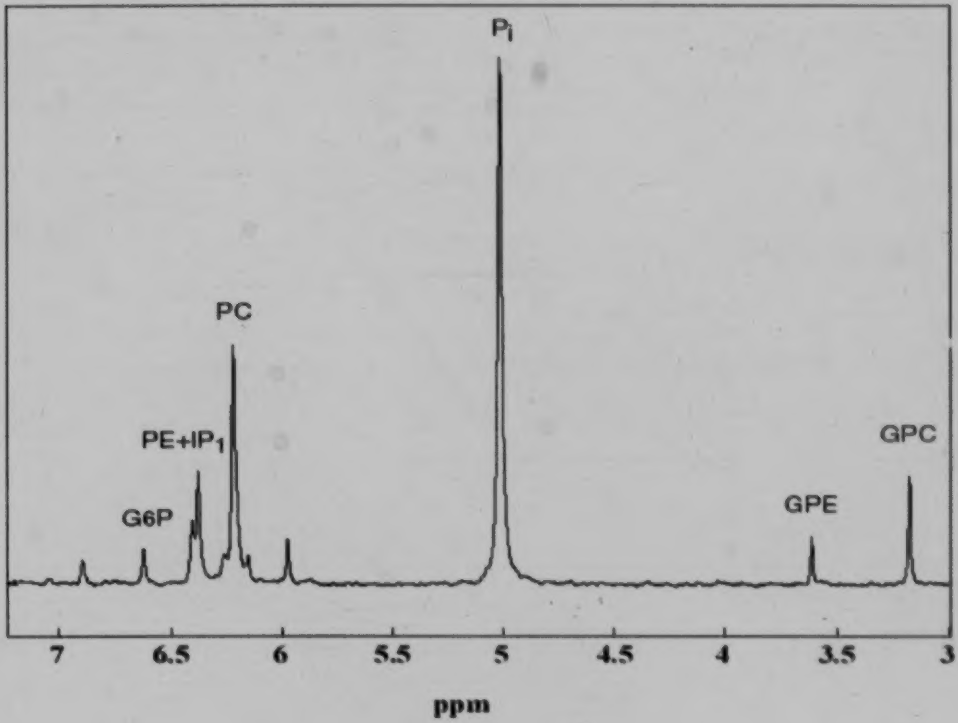

Figure 9: 202.3MHz ^{31}P NMR Spectrum of Rat Brain Extract Focussed Between 3.0 and 7.25 ppm. G6P= glucose-6-phosphate; PE+IP_1= phosphoethanolamine+inositol monophosphates; PC=phosphocholine; P_i=inorganic phosphate; GPE=glycerophosphoethanolamine; GPC=glycerphosphocholine. Adapted from O'Donnell et al., 2000.

Figure 10 and Table 5 illustrate the brain concentrations of IP_1+PE calculated from the observed peak following chronic lithium or sodium valproate administration. As with *myo*-inositol, there was a significant group effect with IP_1+PE due to chronic drug treatment (F=17.315, df=2, p<0.001). Tukey *post-hoc* results showed that both lithium- (p<0.001) and valproate-treated (p<0.001) rats exhibited significantly increased IP_1+PE whole brain concentrations compared to saline-treated rats. *d*-Amphetamine had no effect on IP_1+PE concentrations (F=0.880, df=1, p<0.36).

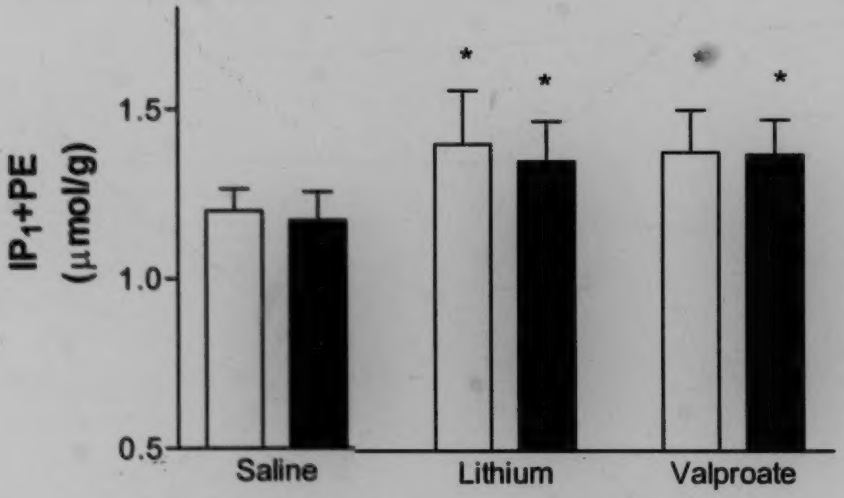

Figure 10: Brain IP_1+PE Concentrations Following Chronic Lithium and Valproate Treatment and Acute d-Amphetamine Administration to Rats. Values represent mean±SEM [n=9]. ■ *=d-amphetamine;* □ *= no d-amphetamine; * = statistically significant versus saline-saline-treated animals. Adapted from O'Donnell et al., 2000.*

No significant changes were found with G6P (F=0.642, df=2, p<0.53), PC (F=2.322, df=2, p<0.62), GPE (F=1.910, df=2, p<0.16), or GPC (F=2.491, df=2, p<0.10). Whole brain concentrations of these compounds following chronic saline or drug administration are illustrated in Table 5. *d*-Amphetamine had no effect on PC (F=0.263, df=1, p<0.11), GPE (F=0.014, df=1, p<0.91), or GPC (F=0.089, df=1, p<0.77) concentrations, but did have a significant effect on G6P (F=5.035, df=1, p<0.030). However, neither lithium or valproate treatment caused significant effects on G6P concentrations.

Table 5: Concentrations of Metabolites in Rat Brain Following Chronic Treatment.

Metabolite	**Saline (μmol/g)**	**Lithium (μmol/g)**	**Valproate (μmol/g)**
Cr+PCr	10.56±0.40	9.65±0.38*	9.84±0.37*
Myo-inositol	7.28±0.31	6.60±0.33*	6.63±0.17*
NAA	8.20±0.40	7.47±0.40*	7.26±0.24*
Glycine	1.16±0.14	1.12±0.06	1.16±0.08
IP_1+PE	1.20±0.07	1.39±0.16*	1.39±0.12*
G6P	0.226±0.021	0.217±0.013	0.222±0.019
GPC	0.631±0.241	0.525±0.065	0.502±0.049
GPE	0.261±0.052	0.224±0.027	0.237±0.012
PC	2.55±0.11	2.60±0.18	2.65±.0.11
Myo-inositol/ (Cr+PCr)	0.699±0.021	0.688±0.037	0.680±0.027

*Values represent the mean±SEM. Concentrations were calculated using the internal standards, TSP, in high-resolution (500MHz) ^1H NMR spectra and DSS, in high resolution (202.3MHz) ^{31}P NMR spectra. * = significantly different ($p<0.05$) from saline-treated rats. Adapted from O'Donnell et al., 2000.*

B. Study 2: The Use of NMR Spectroscopy and HPLC to Study the Effects of Lithium and Valproate on Amino Acid Neurotransmitter Concentrations

B.1. ^{1}H NMR Spectroscopy Results

Using ^{1}H NMR, several significant changes in whole brain metabolites were noted with both lithium- and sodium valproate-treated rats. The concentrations of alanine, aspartate, GABA, glutamate, and taurine were calculated from the peak areas of multiplets at chemical shifts of 1.48, 2.82, 2.31, 2.36, and 3.44 ppm, respectively [Behar and Ogino, 1991]. Whole brain concentrations (μmol/g wet weight) of these metabolites after chronic lithium, sodium valproate, or saline administration are listed in Table 6. Significant drug effects of lithium and sodium valproate were observed with alanine ($F=9.441$, $df=5$, $p<0.001$), aspartate ($F=6.449$, $df=5$, $p<0.001$), GABA ($F=4.068$, $df=5$, $p<0.005$), glutamate ($F=5.282$, $df=5$, $p<0.005$), glycine ($F=1.726$, $df=5$, $p<0.15$) and taurine ($F=3.645$, $df=5$, $p<0.01$).

Dunnett's t-tests revealed that lithium-treated rats exhibited significantly decreased concentrations of aspartate ($p<0.001$), glutamate ($p<0.001$), and taurine ($p<0.001$). Sodium valproate-treated rats exhibited significantly decreased concentrations of alanine ($p<0.001$), aspartate ($p<0.005$), GABA ($p<0.005$), glutamate ($p<0.005$), and taurine ($p<0.05$). Figures 11, 13, 15, 17, 19, and 21 graphically illustrate the effects of lithium and valproate on brain concentrations of GABA, glutamate, glycine, aspartate, alanine, and taurine, respectively as measured by high-resolution NMR spectroscopy.

Acute *d*-amphetamine administration had no significant group effects on alanine (F=1.494, df=1, $p<0.23$), aspartate (F=2.508, df=1, $p<0.13$), GABA (F=0.001, df=1, $p<0.99$), glutamate (F=3.820, df=1, $p<0.06$), or taurine (F=0.533, df=1, $p<0.47$).

B.2. HPLC Results

Whole brain concentrations of alanine, aspartate, GABA, glutamate, and taurine are listed in Table 5. Also shown in Table 5 is a comparison of the observed significant and non-significant changes using NMR to those found using HPLC. Significant drug effects of lithium and sodium valproate were observed with alanine (F=6.200, df=2, $p<0.01$), aspartate (F=4.104, df=2, p<0.03), GABA (F=4.193, df=2, p<0.03), and taurine (F=4.950, df=2, p<0.02).

Dunnett's 2-sided t-test results showed that lithium-treated rats exhibited significantly decreased concentrations of aspartate (p<0.03). Also, decreases in the concentration of taurine (p<0.061) approached significance following chronic lithium treatment. Sodium valproate-treated rats had significantly decreased concentrations of alanine (p<0.005), GABA (p<0.02), and taurine (p<0.01). Aspartate (p<0.054) and glutamate (p<0.065) concentrations also exhibited a decreasing trend following chronic valproate treatment, but these decreases did not reach statistical significance. Figures 12, 14, 16, 18, 20, and 22 graphically illustrate the effects of lithium and valproate on brain concentrations of GABA, glutamate, glycine, aspartate, alanine, and taurine, respectively, as measured by HPLC.

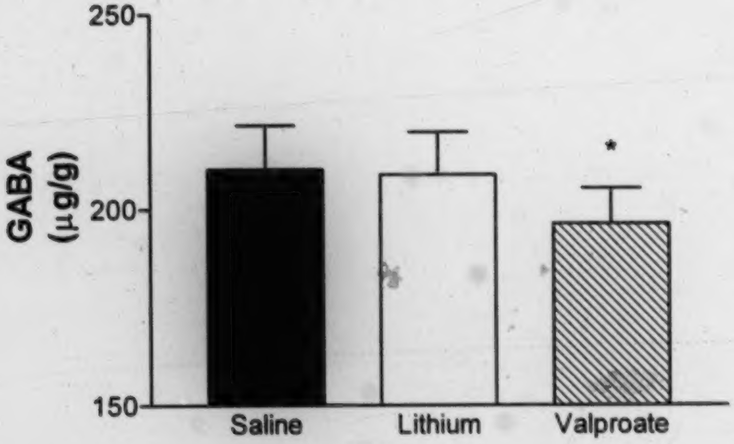

Figure 11: Rat Brain Concentrations of GABA Following Chronic Treatment with Saline, Lithium, or Valproate as Measured by High-resolution NMR Spectroscopy. Values represent mean±SEM [n=18]. * *= statistically significant concentration change compared to saline-treated rats.*

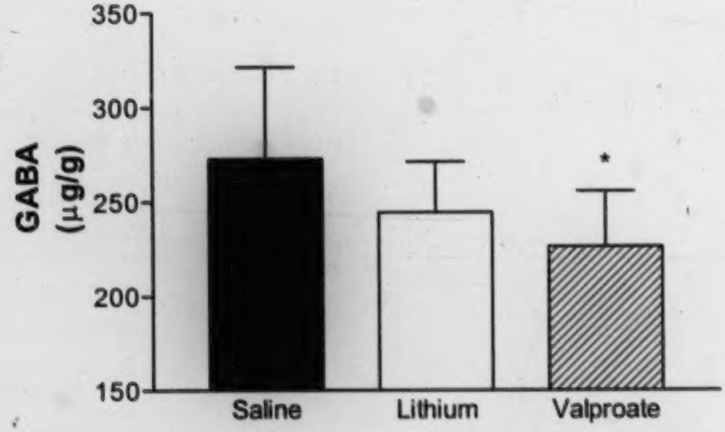

Figure 12: Rat Brain Concentrations of GABA Following Chronic Treatment with Saline, Lithium, or Valproate as Measured by HPLC. Values represent mean±SEM [n=10]. * *= statistically significant concentration change compared to saline-treated rats*

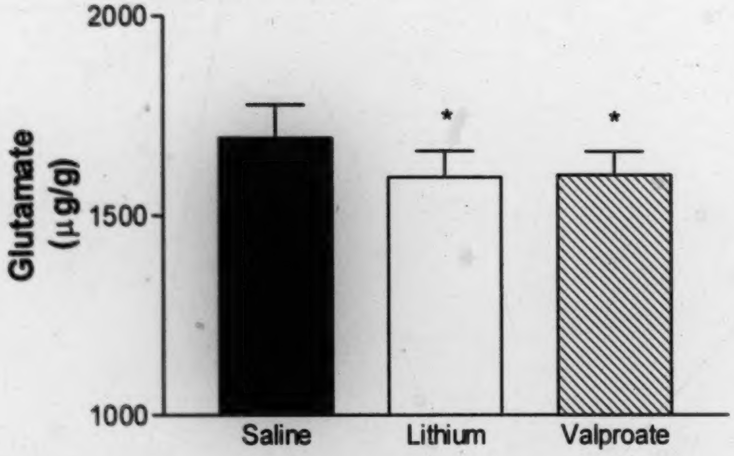

*Figure 13: Rat Brain Concentrations of Glutamate Following Chronic Treatment with Saline, Lithium, or Valproate as Measured by High-resolution NMR Spectroscopy. Values represent mean±SEM [n=18]. * = statistically significant concentration change compared to saline-treated rats*

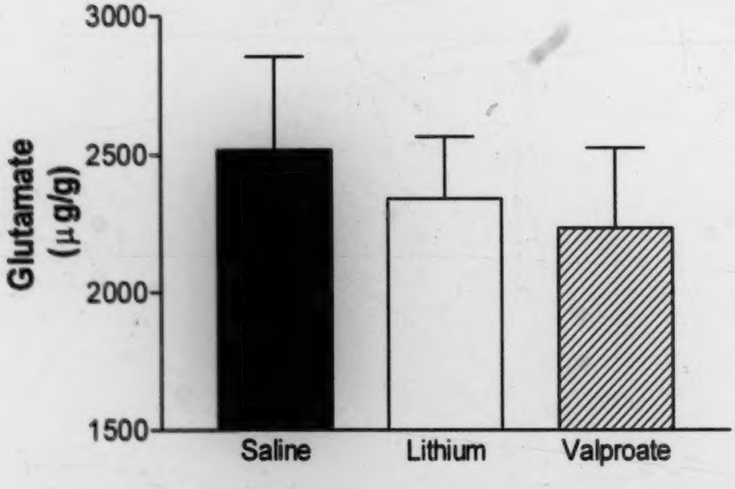

Figure 14: Rat Brain Concentrations of Glutamate Following Chronic Treatment with Saline, Lithium, or Valproate as measured by HPLC. Values represent mean±SEM [n=10].

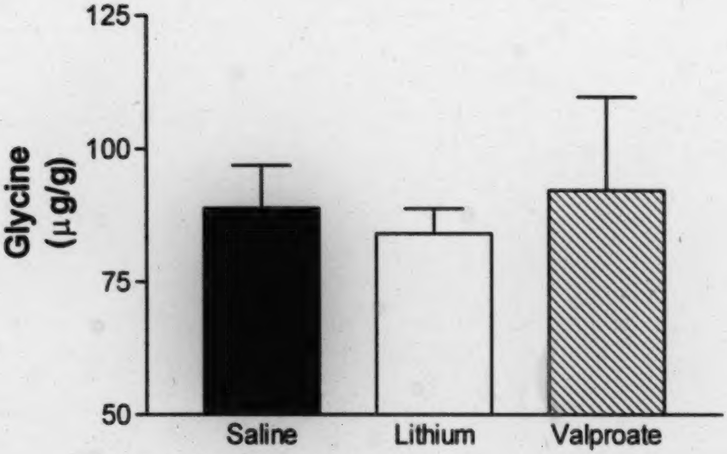

Figure 15: Rat Brain Concentrations of Glycine Following Chronic Treatment with Saline, Lithium, or Valproate as Measured by High-resolution NMR Spectroscopy. Values represent mean±SEM [n=18].

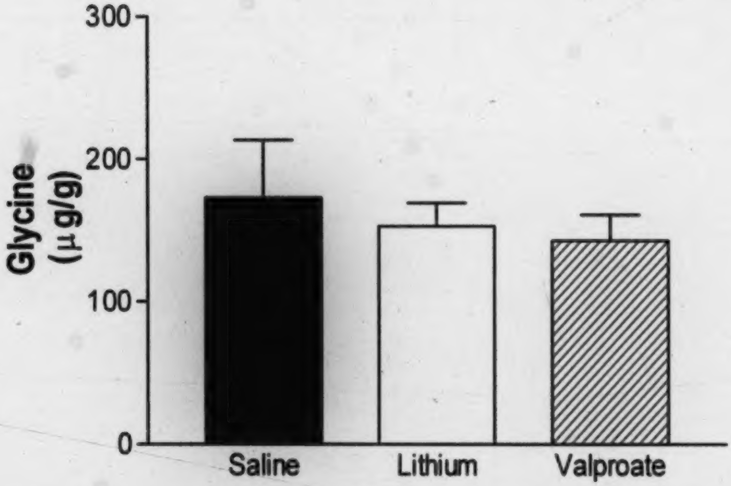

Figure 16: Rat Brain Concentrations of Glycine Following Chronic Treatment with Saline, Lithium, or Valproate as Measured by HPLC. Values represent mean±SEM [n=10].

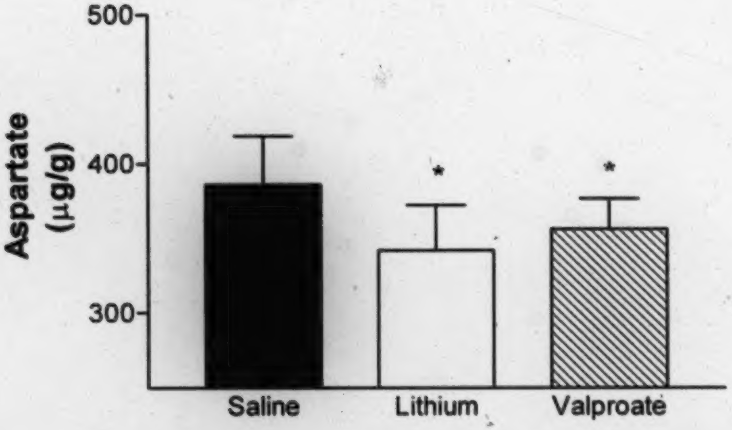

*Figure 17: Rat Brain Concentrations of Aspartate Following Chronic Treatment with Saline, Lithium, or Valproate as Measured by High-resolution NMR Spectroscopy. Values represent mean±SEM [n=18]. * = statistically significant concentration change compared to saline-treated rats*

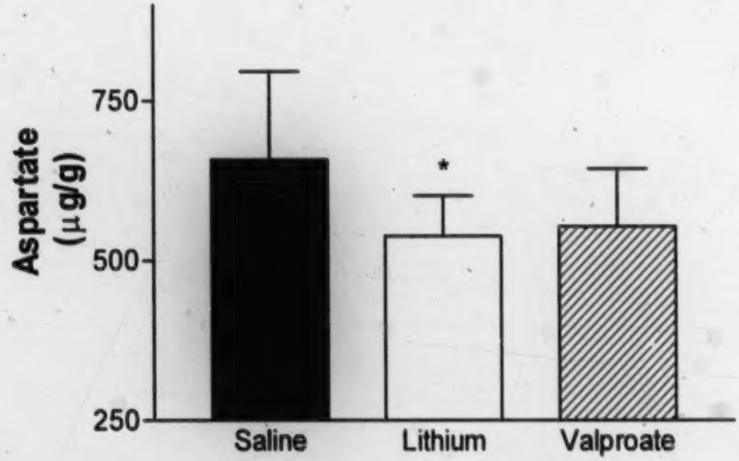

*Figure 18: Rat Brain Concentrations of Aspartate Following Chronic Treatment with Saline, Lithium, or Valproate as Measured by HPLC. Values represent mean±SEM [n=10]. * = statistically significant concentration change compared to saline-treated rats*

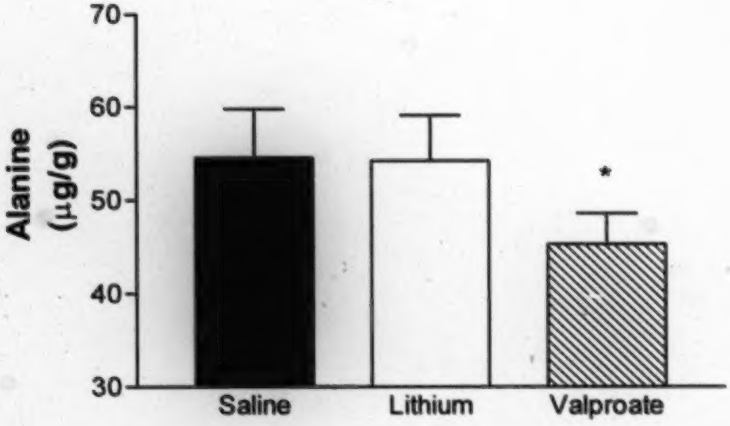

*Figure 19: Rat Brain Concentrations of Alanine Following Chronic Treatment with Saline, Lithium, or Valproate as Measured by High-resolution NMR Spectroscopy. Values represent mean±SEM [n=18]. * = statistically significant concentration change compared to saline-treated rats*

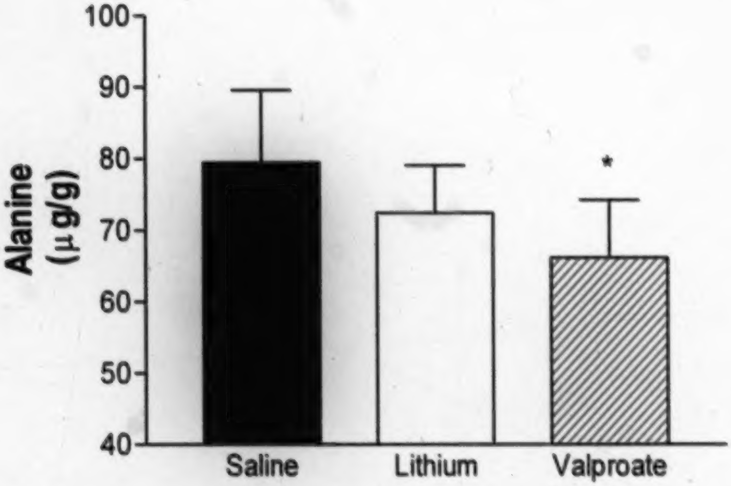

*Figure 20: Rat Brain Concentrations of Alanine Following Chronic Treatment with Saline, Lithium, orValproate as Measured by HPLC. Values represent mean±SEM [n=10]. * = statistically significant concentration change compared to saline-treated rats*

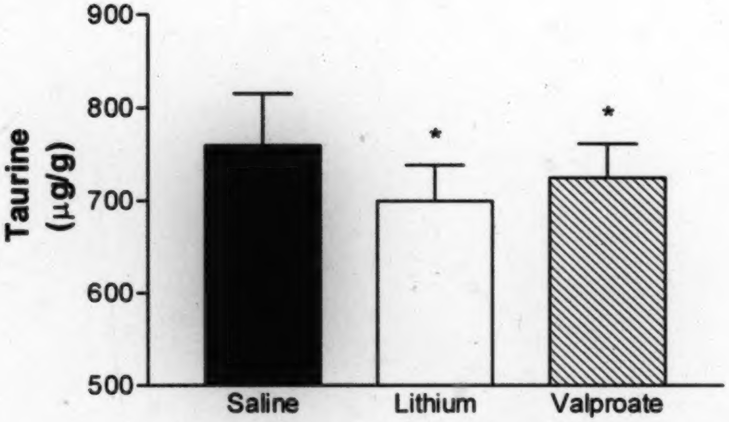

*Figure 21: Rat Brain Concentrations of Taurine Following Chronic Treatment with Saline, Lithium, or Valproate as Measured by High-resolution NMR Spectroscopy. Values represent mean±SEM [n=18]. * = statisticallysignificant concentration change compared to saline-treated rats*

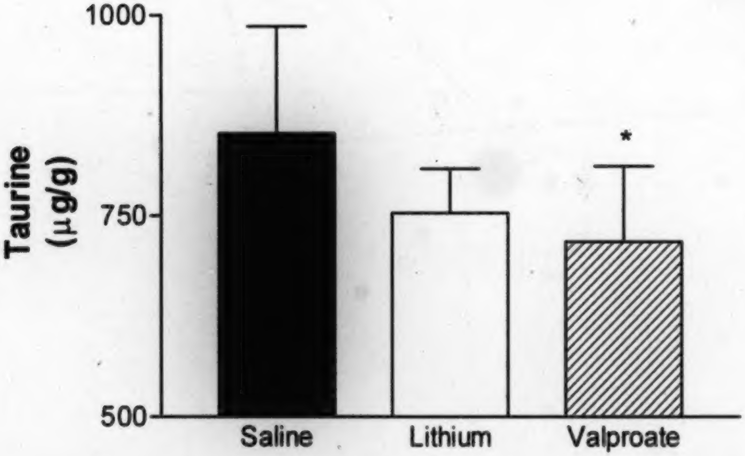

*Figure 22: Rat Brain Concentrations of Taurine Following Chronic Treatment with Saline, Lithium, or Valproate as Measured by HPLC. Values represent mean±SEM [n=10]. * = statistically significant concentration change compared to saline-treated rats*

Table 6: Comparison of NMR Spectroscopy and HPLC in Quantifying Amino Acid Neurotransmitters.

		NMR Analysis	**HPLC Analysis**
Amino Acid	**Drug Group**	**[ ] Change***	**[ ] Change***
Alanine	Saline		
	Lithium	↔	↔
	Valproate	⇓	⇓
Aspartate	Saline		
	Lithium	⇓	⇓
	Valproate	⇓	↓
GABA	Saline		
	Lithium	↔	↔
	Valproate	⇓	⇓
Glutamate	Saline		
	Lithium	⇓	↓
	Valproate	⇓	↓
Glycine	Saline		
	Lithium	↔	↔
	Valproate	↔	↓
Taurine	Saline		
	Lithium	⇓	↓
	Valproate	⇓	⇓

** = Change in amino acid concentrations ([ ]) of drug-treated versus saline treated rats. ↔ = no change; ⇓ = significant decrease; ↓ = non-significant decrease*

Discussion

A. Part I: The Use of NMR/MR Spectroscopy to Study the Inositol-Depletion Hypothesis in Brain Extracts and Human Subjects

A.1. Effects of Lithium and Valproate on *Myo*-inositol and Inositol Monophosphates

The inositol-depletion hypothesis was originally developed to explain lithium's therapeutic efficacy following evidence of IMPase inhibition. In the present study, it has been shown for the first time that chronic therapeutic doses of lithium and valproate have similar effects on the PI-cycle by decreasing the concentration of *myo*-inositol and increasing the concentration of the inositol monophosphates in whole rat brain. Since only lithium has been shown to inhibit IMPase [Hallcher and Sherman, 1980; Naccarato et al., 1974; Nahorski et al., 1991], it is unlikely that these two drugs elicit the observed changes by the same mechanisms. However, the observations are interesting nonetheless, since lithium and valproate appear to have similar effects on the concentrations of two crucial metabolites in the PI-cycle.

This study attempted to overcome several of the methodological obstacles confounding previous research pertaining to the effects of lithium and valproate on the PI-cycle. By chronically treating rats with doses producing therapeutic plasma levels reflective of those found in patients, only physiological changes that are induced by therapeutic treatment should be observed. Also, relatively high numbers of animals (n=18/chronic treatment group) were employed in this study to ensure that if statistically significant changes were occurring, they would be detected. It is important to note that the reported concentration values were not corrected for recoveries. Finally, because this study isolated and measured concentrations of *myo*-inositol and inositol monophosphates in brain tissue using a relatively simple method, the complications of using assays involving radiolabelling do not become an issue. However, due to the fact that NMR spectroscopy suffers from poor sensitivity and the phosphorus compounds were of low concentrations, whole brain was used. Thus, this study did not address the possible existence of region-specific concentration changes in *myo*-inositol and the inositol monophosphates due to lithium administration.

While this is the first time valproate has been shown to have effects conforming to the inositol-depletion hypothesis, previous studies have described effects of valproate on the PI-cycle. Incubation of mouse brain slices with sodium valproate was shown to lead to an accumulation of Ins(1,4,5)P_3, but not inositol monophosphates [Dixon and Hokin, 1997]. Another study found a significant attenuation of striatal agonist (carbachol or norepinephrine)-stimulated inositol phosphate formation following chronic sodium valproate treatment, suggesting effects of chronic sodium valproate on PI-cycle functioning [Li et al., 1993]. While these findings appear to be inconsistent with the results of the present study, methodological differences may contribute to discrepancies between the studies. As described earlier, the use of agonist stimulation and radiolabelling may result in misleading results when investigating the inositol-depletion hypothesis.

At present, the mechanism by which valproate decreases the concentration of *myo*-inositol and increases inositol monophosphate concentrations is unclear and requires further research. With lithium, these changes can be easily correlated to an inhibition of IMPase. Since valproate has been previously shown to have no effect on IMPase [Vadnal and Parthasarathy, 1995], these changes are not easily explained. The therapeutic relevance of these observations is also difficult to discern. While lithium's therapeutic mechanism of action is generally associated with its inhibition of IMPase and associated downstream effects on second messenger systems, no direct effects of valproate on the PI-cycle have been identified. Thus, these changes may be the result of an indirect mechanism leading to disturbances in the production of PI-cycle-associated second messengers. However, the short- and long-term effects of disruptions in the PI-cycle have not been yet correlated with the alleviation of clinical bipolar symptoms. Thus, further research is required to interpret the therapeutic relevance of altered PI-cycle functioning.

These findings add to other research suggesting that lithium and valproate may have common effects on signal transduction pathways. Recently, Manji and colleagues [2000] carried out an extensive review of this literature. Interestingly, both lithium and sodium valproate have been shown to have similar effects on the PKC family of enzymes which are closely associated with PI-cycle functioning. Also, both drugs have been implicated in the regulation of glycogen synthase kinase 3β and activator protein 1 (AP-1) transcription factor [Jope et al., 2000]. However, the mechanisms by which these changes occur have not yet been elucidated, and further research is required.

A.2. The Effects of *d*-Amphetamine on the PI-cycle

In contrast to our hypothesis regarding the stimulation of the PI-cycle, *d*-amphetamine did not potentiate the observed effects of lithium and valproate in the present study. It is possible that the dose administered or the length of time between injection and sacrifice of animals was not optimal for observing effects of *d*-amphetamine on concentrations of *myo*-inositol and the inositol monophosphates. Thus, *d*-amphetamine's effects on the PI-cycle must be assessed in a time- and concentration-dependent manner in future work.

A.3. Quantification Issues in Examining the Inositol-Depletion Hypothesis: Significance of Present Findings in *In Vivo* ^{1}H and ^{31}P MRS Studies

While more sensitive quantification methods for the measurement of *myo*-inositol and inositol monophosphates have been developed, the use of high resolution (11.8T) NMR was advantageous in this study for several reasons. First, the use of quantitative high-resolution NMR spectroscopy allowed the simultaneous monitoring of compounds observed in the poorly resolved human MRS spectra. Second, a number of compounds that cannot be resolved using *in vivo* ^{31}P and ^{1}H MRS were resolved using high-resolution NMR spectroscopy. Thus, peaks that are co-resonant in *in vivo* human MRS spectra were separated, and the effects of drugs on compounds that normally interfere with the metabolites of interest could be assessed. For example, in ^{1}H spectra, glycine was isolated from the *myo*-inositol peak while in ^{31}P spectra many of the peaks contributing to the PME peak in human ^{31}P MRS spectra were separated and assessed separately. These findings will be discussed later in this section. Finally, the use of quantitative high-resolution NMR spectroscopy allowed the assessment of lithium and valproate's effects on Cr+PCr and NAA peaks. In the past, these peaks have been used as internal references in *in vivo* human ^{1}H MRS studies of the inositol-depletion hypothesis.

A.3.1. Ratio Data Expression

The use of internal references and ratio data expression in human MRS studies is usually employed where *in vivo* absolute quantitative techniques are not available. In ^{1}H MRS studies, both the Cr+Pr and NAA peaks have been used as the internal references against which peaks of interest are compared. However, we have demonstrated that this method of data expression is inappropriate when lithium or sodium valproate are administered chronically. Both drugs caused a significant decrease in Cr+PCr concentrations in whole rat brain following chronic administration.

Therefore, in *in vivo* ^{1}H MRS studies where lithium or sodium valproate is administered to patients and controls, the use of Cr+PCr as an internal reference may lead to results that are not reflective of changes in the metabolite of interest. Significant changes in these ratios may reflect drug-dependent alterations in Cr+PCr concentrations rather than those in he compound of interest. Also, non-significant changes may result when changes in the numerator (*myo*-inositol) and the denominator (Cr+PCr) are on the same order of magnitude. This is effectively illustrated in Table 4 and Figure 23 where ratios of *myo*-inositol/(Cr+PCr) from the present study are shown to be non-significant.

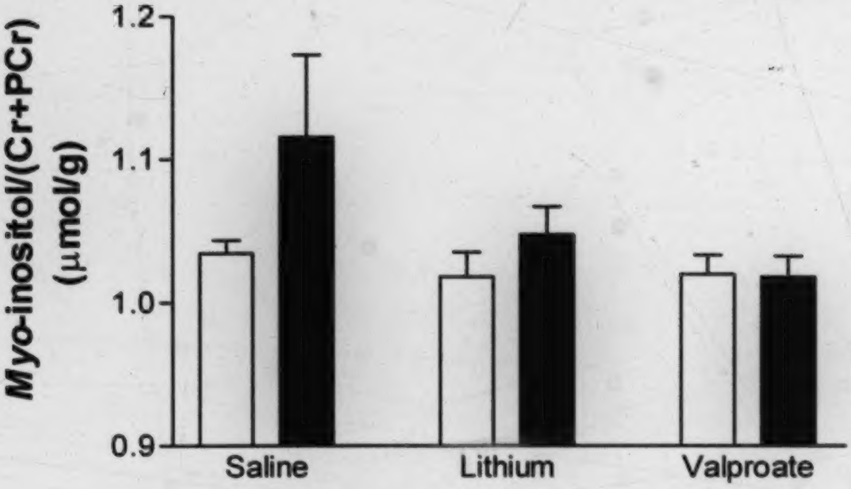

Figure 23: Myo-inositol/(Cr+PCr) Ratios Following Chronic Lithium and Sodium Valproate Administration. No concentration changes were observed in brain myo-inositol when the present data were presented as a ratio of myo-inositol/(Cr+PCr). The Cr+PCr peak is a commonly used internal reference in human in vivo ^{1}H MRS research. ■ *= d-amphetamine;* □ *= no d-amphetamine; Values represent mean±SEM [n=9].*

Because it is found primarily in neurons, NAA is often used as a neuronal marker in MRS research [Birken and Oldendorf, 1989]. While there is evidence that a decrease in NAA correlates well with a decrease in neuronal density [Ebisu et al., 1994; Nakano et al., 1998], it is not known whether acute or chronic drug treatment can alter the concentration of NAA in the absence of neuronal death. In the present study, concentrations of NAA were found to decrease following chronic treatment with either lithium or valproate in rat brain. However, it is not known whether these changes were due to neuronal death or a disruption of NAA homeostasis caused by lithium and valproate administration. Thus, caution must be exercised when interpreting decreases in the concentration of NAA as cell death. Further research is required to identify the circumstances under which NAA concentrations are altered and the mechanisms by which this occurs.

In terms of *in vivo* ^{1}H MRS research and their use as internal references, the present study has raised concerns about Cr+PCr and NAA that need to be addressed. Further research is required to examine the effects of lithium and valproate on the Cr+PCr and NAA peaks in bipolar patients and healthy controls. The present findings with creatine and NAA suggest that future *in vivo* ^{1}H MRS research on lithium- and sodium valproate-treated subjects must employ quantification methods versus ratio data expression if the results obtained are to be reliable. Thus, in past *in vivo* ^{1}H MRS studies where lithium or valproate has been administered chronically, caution must be exercised in the interpretation of these data. Ratios presented in these studies may reflect changes in both the compound of interest and the compound used as the reference, leading to misleading results.

The practicality of using internal references in ^{31}P MRS spectra could not be assessed in the present study because the extraction method used to prepare samples for NMR spectroscopy did not adequately extract a number of compounds from rat brain that are observed in *in vivo* ^{31}P MRS spectra of humans. However, given the observations with the Cr+PCr and NAA peaks, caution must be exercised when employing ratio methods of data expression. Ideally, the effects of drug administration and psychiatric state should be assessed before a given compound is used as an internal reference.

A.3.2. Co-resonance of Compounds and the Inositol-depletion Hypothesis

Using human ^{1}H MRS, the peaks at 3.54 and 3.62 ppm are often used to measure *myo*-inositol concentrations *in vivo*. Glycine, inositol monophosphates, and *myo*-inositol all co-resonate in the peak at 3.54 ppm while *myo*-inositol and inositol monophosphates co-resonate at 3.62 ppm. In the present study, using high-resolution ^{1}H (11.8T) NMR, *myo*-inositol and the inositol monophosphates could not be resolved. However, using ^{31}P NMR, the concentration of IP_1+PE was found to be much lower than the calculated concentration of *myo*-inositol in ^{1}H NMR spectra. Therefore, the inositol monophosphates are assumed to contribute a negligible signal to the *myo*-inositol peak in ^{1}H NMR spectra. Also, no concentration changes in glycine were found with either lithium or valproate. Thus, it is likely that changes observed in the "*myo*-inositol" peaks of *in vivo* ^{1}H MR spectra are reflective of concentration changes in *myo*-inositol rather than in the inositol monophosphates or glycine.

In ^{31}P NMR spectra of the present study, several compounds contributing to the PME peak of *in vivo* ^{31}P MR spectra were resolved into separate peaks. The concentration of PC, one of the major contributors to the PME peak, was not affected by lithium or valproate administration in rat brain. G6P was also unaffected by lithium and valproate administration. Thus, lithium- and valproate-induced changes in the PME peak of *in vivo* ^{31}P MR spectra are likely not due to either PC or G6P. However, since PE cannot be resolved from the inositol monophosphates, the possibility still exists that lithium or valproate may affect the concentration of PE.

A.4. Part I: Summary of Findings

Quantitative high resolution (11.8T) ^{1}H and ^{31}P NMR spectroscopy were employed in this study to simultaneously assess changes in neurochemicals following chronic lithium or valproate treatment in rat brain. The neurochemicals examined were those which have been found to be important when *in vivo* ^{1}H and ^{31}P MRS is used to study the effects of mood stabilizers in bipolar patients. Several important observations were made: (1) Chronic lithium and valproate have similar effects on the concentrations of *myo*-inositol and inositol monophosphates in whole rat brain. (2) The concentrations of both Cr+PCr and NAA, two commonly used internal references in human ^{1}H MRS, decreased in rat brain following chronic administration of lithium or valproate. (3) Compounds that co-resonate with *myo*-inositol and the inositol monophosphates in human ^{1}H and ^{31}P MR spectra, respectively did not change in rat brain following lithium or valproate administration. (4) Acute *d*-amphetamine did not affect the concentrations of *myo*-inositol and inositol monophosphates in rat brain.

B. Part II: The Use of NMR spectroscopy to Study the Effects of Lithium and Valproate on Amino Acid Concentrations

B.1. The Use of NMR Spectroscopy to Measure Amino Acids

In the present study, two different procedures were used to measure the concentrations of several amino acid neurotransmitters in whole rat brain following chronic administration of either lithium or sodium valproate. Amino acid concentrations were determined using either ^{1}H NMR spectroscopy or HPLC.

The results of this study showed that chronic administration of either lithium and valproate led to similar changes in whole brain concentrations of several important amino acids. However, measurement of brain amino acids by high-resolution NMR and HPLC did not lead to consistent significant concentration changes for each individual amino acid following drug treatment. Nevertheless, many of the same trends in the concentrations of each amino acid were observed between the methods and thus we can conclude that high-resolution NMR is a viable tool for brain amino acid research. However, in the present study, the NMR results were not corrected for recoveries, thus explaining the variation in whole brain concentrations of individual amino acids using NMR and HPLC. Finally, the fact that similar changes in whole brain concentrations reached statistical significance in the NMR group, but only presented as a trend in the HPLC group may be explained by the larger group size in the NMR study (n=18 versus n=10 HPLC analysis).

It is important to note that differences between the present findings and previously published studies may be attributed in large part to methodological differences. Thus we measured the concentration of each neurochemical in whole brain while most studies measured concentrations in specific brain regions. Thus it may be difficult to compare our results to these studies as changes in a given amino acid may vary from region to region. It is also possible that such regional changes may go undetected when whole brain is examined.

B.2. GABA

Using NMR and HPLC, a decrease in the concentrations of whole brain GABA was found following chronic treatment with sodium valproate in both groups of rats. The present finding that whole brain GABA concentrations decreased after chronic sodium valproate administration is in contrast to previous research in this area. A number of studies, discussed in the Introduction of this thesis have found that GABA increases in specific brain regions following valproate administration. One reason our results differed from much of the previous literature may be the fact that the present study measured GABA concentrations in whole brain rather than in specific brain regions or nerve terminals. While regional increases are observed, the net effect of valproate on whole brain may be to decrease the total concentration of GABA. Thus more research needs to be completed to fully elucidate the effects of valproate on the GABA system in brain tissue.

In the present study, chronic lithium did not appear to affect the concentration of GABA in whole rat brain. This finding is supported by another study which also found no significant changes in the concentration of GABA in the cerebral cortex, hippocampus, striatum or substantia nigra following lithium treatment [Jope et al., 1989]. However, other studies have found lithium to increase the concentration of GABA in a number of brain regions following both acute and chronic administration [Marcus et al., 1986; Gottesfeld, 1976]. Thus the effects of lithium on the GABA system are not completely clear at present.

B.3. Glutamate

Lithium and sodium valproate both caused a significant reduction in the whole brain concentration of the excitatory neurotransmitter, glutamate, when concentrations were measured with NMR. When HPLC was used however, a non-significant decreasing trend in glutamate concentrations was observed with both drugs. While we found a trend towards a decrease in whole brain glutamate following valproate administration, no effects of valproate on regional brain glutamate have been found overall by other authors. However, since the present study examined whole brain, there may be a net decrease in the concentration of glutamate in whole brain that is too small to be observed in individual brain regions.

As with valproate, there was a decrease in whole brain glutamate concentrations following chronic lithium administration. This finding is supported by a study which reported a similar decrease in brain glutamate [Plenge, 1976]. Other studies, described in the Introduction, have found increases in the concentration of glutamate. However, these studies examined individual brain regions rather than whole brain and thus the effects of lithium may vary from region to region, resulting in an overall net change in the concentration of glutamate.

Finally, while the HPLC method resulted in a better recovery of glutamate, the calculated concentrations in the present rat brains were higher than values previously reported by other workers in the Neurochemical Research Unit of the Department of Psychiatry at the University of Alberta (Paslawski et al., 1999). However, more recent work in the Neurochemical Research Unit suggests that there may be seasonal changes in brain concentrations of glutamate (Parent and Baker, personal communication). Thus, the potential relationship between brain glutamate concentrations and seasonal variation must be assessed in future work.

B.4. Aspartate

The whole brain concentration of aspartate was reduced by chronic treatment with both lithium and sodium valproate. Concentration measurements of aspartate by NMR gave results that were statistically significant for both drug groups. Using HPLC, however, only lithium-treated rats exhibited significantly decreased concentrations of aspartate while valproate-treated rats showed a trend towards decreased concentrations that approached statistical significance ($p<0.054$). This reduction is consistent with previous research examining the effects of valproate [Chapman et al., 1982; Löscher and Hörstermann, 1994] and lithium [Jope et al., 1989] on brain aspartate concentrations. Thus, the evidence supports common effects of both lithium and valproate to decrease aspartate concentrations.

B.5. Taurine

Using NMR, the whole brain concentrations of taurine were significantly reduced by chronic administration of lithium. Using HPLC, however, there was a trend for whole brain concentrations of taurine to decrease but this decrease did not reach statistical significance ($p<0.061$). In contrast to our results, a previous study examining the effects of lithium on regional brain taurine showed no significant changes in the cerebral cortex, hippocampus, striatum, or substantia nigra following chronic treatment [Jope et al., 1989]. Valproate-treated rats showed significantly decreased taurine levels using both NMR and HPLC. However, previous studies have shown valproate to increase taurine in whole brain [Patsalos and Lascelles, 1981]; regional studies demonstrated this increase was particularly marked in the hippocampus [Löscher and Hörstermann, 1994]. Thus, at present there is no clear consensus on the effects of lithium and valproate on brain taurine concentrations.

B.6. Alanine

In the present study, we found that lithium had no significant effects on the concentration of alanine as measured by both NMR and HPLC. A previous study showed a significant increase in alanine levels in the substantia nigra following chronic administration, while no changes were observed in the cerebral cortex, hippocampus, or striatum [Jope et al, 1989]. Thus it is possible that in the present assays, any regional changes have been masked by using whole brain. Sodium valproate caused a significant decrease in the concentration of brain alanine compared to saline-treated rats as measured by both NMR and HPLC. The effect of valproate on alanine has not been previously studied.

It has been suggested that alanine acts as nitrogen carrier between astrocytes and glutaminergic neurons where ammonia generated by the glutaminase reaction in glutaminergic neurons is shuttled to astrocytes to be used in glutamine synthesis [Waagepetersen et al., 2000]. Furthermore, since alanine synthesis is closely linked to glucose metabolism, concentration changes caused by drugs such as valproate may be occurring as a result of altered energy metabolism. However, further research is required to elucidate the mechanisms underlying the observed concentration changes in alanine in the present experiment.

B.7. Effects of *d*-Amphetamine on Amino Acid Neurotransmitters

Acute *d*-amphetamine did not have statistically significant effects on any of the concentrations of brain amino acids measured using high resolution NMR. Other studies have found that amphetamine increases extracellular glutamate [Karler et al., 1994; Reid et al., 1997]. However, because whole brain concentrations were measured in the present study, we had no way of differentiating between intracellular and extracellular concentrations of amino acids. Thus, if there was no net change in the combined intracellular and extracellular concentration of a given amino acid, no significant differences would be detected. Therefore, this model was not used further in the rats prepared for the HPLC analyses, and the acute injection of *d*-amphetamine or saline before decapitation was eliminated.

B.8. Part II: Summary of Findings

High-resolution ^{1}H NMR spectroscopy was shown to be an effective tool for measuring amino acid neurotransmitters in rat brain. This method was validated by using HPLC and NMR to quantify amino acid neurotransmitters in rat brain following chronic treatment with lithium or valproate in two separate experiments. Both methods identified similar trends in the concentrations of each amino acid following drug treatment.

Lithium and valproate were found to have both similar and dissimilar effects on the concentrations of amino acids in whole rat brain following chronic administration. Both drugs elicited similar decreases in the concentrations of glutamate, aspartate, and taurine in whole rat brain. Also, the concentration of glycine did not change with either drug. However, the concentrations of GABA and alanine were found to decrease in whole rat brain following valproate treatment while lithium was found to have no effects on these two amino acids.

C. Overall Conclusion

Lithium and valproate appear to have common effects on the concentrations of a number of brain chemicals that may be related to their therapeutic mechanisms of actions. Of particular significance are the common effects of lithium and valproate in increasing the concentrations of inositol monophosphates and decreasing the concentrations of *myo*-inositol in rat brain following chronic administration. However, the concentration changes induced by lithium and valproate in the present study have not been correlated to the alleviation of clinical symptoms in the treatment of Bipolar Disorder. Thus, further work is needed to understand the clinical significance of neurochemical concentration changes induced by mood stabilizers.

References

Agam G, Livne A: Inositol-1-phosphatase of human erythrocytes is inhibited by therapeutic lithium concentrations. Psychiatry Res 27:217-224, 1989

Agam G, Balfour N, Shapiro J: Plasma inositol levels in lithium-treated manic-depressives, schizophrenics and controls. Human Psychopharmacology 10:311-314, 1995

Ala-Korpela M, Usenius JP, Keisala J, van den Boogaart A, Vainio P, Jokisaari J, Soimakallio S, Kauppinen R: Quantification of metabolites from single-voxel in vivo 1H NMR data of normal human brain by means of time-domain data analysis. MAGMA 3:129-136, 1995

Allen PS: In vivo magnetic resonance spectroscopy applied to medicine. J Can Assoc Radiol 41:39-44, 1990

Allison JH, Blisner ME, Holland WH, Hipps PP, Sherman WR: Increased brain myo-inositol 1-phosphate in lithium-treated rats. Biochem Biophys Res Commun 71:664-670, 1976

Allison JH, Stewart MA: Reduced brain inositol in lithium-treated rats. Nature [New Biol] 233:267-268, 1971

Atack JR: Inositol monophosphatase, the putative therapeutic target for lithium. Brain Res Brain Res Rev 22:183-190, 1996

Atack JR: Lithium, phosphatidylinositol signaling, and bipolar disorder, in Bipolar Medications: Mechanisms of Action. Edited by Manji HK, Bowden CL, Belmaker RH, Washington DC, American Psychiatric Press Inc, 2000, pp 1-30

Baastrup P, Poulsen KS, Schou M, Thomsen K, Amdisen A: Prophylactic lithium: double blind discontinuation in manic-depressive and recurrent-depressive disorders. Lancet 2:326-330, 1970

Behar KL, Ogino T: Assignment of resonance in the 1H spectrum of rat brain by two-dimensional shift correlated and J-resolved NMR spectroscopy. Mag Reson Med 17:285-303, 1991

Belmaker RH, Agam G, van Calker D, Richards MH, Kofman O: Behavioral reversal of lithium effects by four inositol isomers correlates perfectly with biochemical effects on the PI cycle: depletion by chronic lithium of brain inositol is specific to hypothalamus, and inositol levels may be abnormal in postmortem brain from bipolar patients. Neuropsychopharmacology 19:220-232, 1998

Berridge MJ: Intracellular channels and calcium spiking. Biomed Res 14(suppl 2):21-27, 1993

Berridge MJ: The biology and medicine of calcium signalling. Mol Cell Endocrinol 98:119-24, 1994

Berridge MJ, Downes CP, Hanley MR: Lithium amplifies agonist-dependent phosphatidylinositol responses in brain and salivary glands. Biochem J 206:587-595, 1982

Berridge MJ, Irvine RF: Inositol phosphates and cell signalling. Nature 341:197-205, 1989

Bersudsky Y, Kaplan Z, Shapiro Y, Agam G, Kofman O, Belmaker RH: Behavioral evidence for the existence of two pools of cellular inositol. Eur Neuropsychopharmacol 4:463-467, 1994

Biggs CS, Pearce BR, Fowler LJ, Whitton PS: Regional effects of sodium valproate on extracellular concentrations of 5-hydroxytryptamine, dopamine, and their metabolites in rat brain: an in vivo microdialysis study. J Neurochem 59:1702-1708, 1992

Birken DL, Oldendorf WH: N-acetyl-L-aspartic acid: a literature review of a compound prominent in 1H-NMR spectroscopic studies of brain. Neurosci Biobehav Rev 13:23-31, 1989

Bligh G, Dyer WJ: A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911-917, 1959

Bowden CL: Valproate in mania, in Bipolar Medications: Mechanisms of Action. Edited by Manji HK, Bowden CL, Belmaker RH, Washington DC, American Psychiatric Press Inc, 2000, pp 357-365

Bowden CL, Brugger AM, Swann AC, Calabrese JR, Janicak PG, Petty F, Dilsaver SC, Davis JM, Rush AJ, Small JG, et al: Efficacy of divalproex vs lithium and placebo in the treatment of mania. The Depakote Mania Study Group. JAMA 271:918-924, 1994

Bradford PG, Rubin RP: Quantitative changes in inositol 1,4,5-trisphosphate in chemoattractant-stimulated neutrophils. J Biol Chem 261:15644-15647, 1986

Brammer M, Weaver K: Kinetic analysis of A23187-mediated polyphosphoinositide breakdown in rat cortical synaptosomes suggests that inositol bisphosphate does not arise primarily by degradation of inositol trisphosphate. J Neurochem 53:399-407, 1989

Bruhn H, Stoppe G, Staedt J, Merboldt KD, Hänicke W, Frahm J: Quantitative proton MRS in vivo shows cerebral myo-inositol and cholines to be unchanged in manic-depressive patients treated with lithium. Proc Soc Mag Res Med 1543, 1993

Buchner K: Protein kinase C in the transduction of signals toward and within the cell nucleus. Eur J Biochem 228:211-221, 1995

Cade JF: Lithium salts in the treatment of psychotic excitement. Bull World Health Organ 2000 78:518-520, 1949

Cady EB: Clinical Magnetic Resonance Spectroscopy. New York, Plenum Press,1990

Cao BJ, Peng NA: Magnesium valproate attenuates hyperactivity induced by dexamphetamine- chlordiazepoxide mixture in rodents. Eur J Pharmacol 237:177-181, 1993

Cappeliez P, Moore E: Effects of lithium on an amphetamine animal model of bipolar disorder. Prog Neuropsychopharmacol 14: 347-358, 1990

Casebolt TL, Jope RS: Chronic lithium treatment reduces norepinephrine-stimulated inositol phospholipid hydrolysis in rat cortex. Eur J Pharmacol. 140:245-246, 1987

Cerdan S, Parrilla R, Santoro J, Rico M: 1H NMR detection of cerebral myo-inositol. FEBS Lett 187:167-172, 1985

Chandrasekhar R, Huang HM, Sun GY: Alterations in rat brain polyphosphoinositide metabolism due to acute ethanol administration. J Pharmacol Exp Ther 245:120-123, 1988

Chapman A, Keane PE, Meldrum BS, Simiand J, Vernieres JC: Mechanism of anticonvulsant action of valproate. Progr Neurobiol 19:315-359, 1982

Chen G, Huang LD, Jiang YM, Manji HK: The mood-stabilizing agent valproate inhibits the activity of glycogen synthase kinase-3. J Neurochem 72: 879-882, 1999

Clark JA, Amara SG: Amino acid neurotransmitter transporters: structure, function, and molecular diversity. Bioessays 15:323-332, 1993

Colin SF, Chang HC, Mollner S, Pfeuffer T, Reed RR, Duman RS, Nestler EJ: Chronic lithium regulates the expression of adenylate cyclase and Gi-protein alpha subunit in rat cerebral cortex. Proc Natl Acad Sci USA 88:10634-7, 1991

Danielsen ER, Henriksen O: Absolute quantitative proton NMR spectroscopy based on the amplitude of the local water suppression pulse. Quantification of brain water and metabolites. NMR Biomed 7:311-318, 1994

Diagnostic and Statistical Manual of Mental Disorders: Washington, D.C American Psychiatric Association, Forth Edition, 1994

Deicken RF, Fein G, Weiner MW: Abnormal frontal lobe phosphorus metabolism in bipolar disorder. Am J Psychiatry 152:915-918, 1995a

Deicken RF, Weiner MW, Fein G: Decreased temporal lobe phosphomonoesters in bipolar disorder. J Affect Disord 33:198-199, 1995b

Dixon JF, Hokin LE: A novel action of lithium: stimulation of glutamate release and inositol 1,4,5-triphosphate accumulation via activation of N-methyl D-aspartate receptor in monkey and mouse cerebral cortex slices. Adv Enz Reg 36:229-244, 1996

Dixon JF, Hokin LE: The antibipolar drug valproate mimics lithium in stimulating glutamate release and inositol 1,4,5-triphosphate accumulation in brain cortex slices but not accumulation of inositol monophosphates and bisphosphates. Proc Natl Acad Sci USA 94:4757-4760, 1997

Dixon JF, Hokin LE: Lithium acutely inhibits and chronically up-regulates and stabilizes glutamate uptake by presynaptic nerve endings in mouse cerebral cortex. Proc Natl Acad Sci USA 95:8363-8368, 1998

Dubovsky, SL: Calcium antagonists in manic-depressive illness. Neurophysiology 27:184-192, 1993

Ebisu T, Rooney WD, Graham SH, Weiner MW, Maudsley AA: N-acetylaspartate as an in vivo marker of neuronal viability in kainate-induced status epilepticus: 1H magnetic resonance spectroscopic imaging. J Cereb Blood Flow Metab 14:373-382, 1994

Elphick M, Taghavi Z, Powell T, Godfrey PP: Alteration of inositol phospholipid metabolism in rat cortex by lithium but not carbamazepine. Eur J Pharmacol 156:411-414, 1988

Emrich HM, von Zerssen D, Kissling W, Moller HJ, Windorfer A: Effect of sodium valproate on mania. The GABA-hypothesis of affective disorders. Arch Psychiatr Nervenkr 229:1-16, 1980

Ernst T, Hennig J: Improved water suppression for localized in vivo 1H spectroscopy. J Magn Reson B 106:181-186, 1995

Exton JH: Role of G proteins in activation of phosphoinositide phospholipase C. Adv Second Messenger Phosphoprotein Res 28:65-72. 1993

Farrar TC: Principles of pulse NMR spectroscopy, in NMR: Principles and Applications to Biomedical Research. Edited by Pettegrew JW, New York, Springer-Verlag, 1990, pp 1-36

Fessler RG, Sturgeon RD, London SF, Meltzer HY: Effects of lithium on behaviour induced by phencyclidine and amphetamine in rats. Psychopharmacology 78:323-326, 1982

Fisher SK, Heacock AM, Agranoff BW: Inositol lipids and signal transduction in the nervous system: an update. J Neurochem 58:18-38, 1992

Gani D, Downes CP, Batty I, Bramham J: Lithium and myo-inositol homeostasis. Biochim Biophys Acta 1177:253-269, 1993

Ghaemi SN, Goodwin, FK: Use of atypical antipsychotic agents in bipolar and schizoaffective disorders: a review of empirical literature. J Clin Psychopharmacol 19:354-361, 1999

Ghoshdastidar D, Dutta RN, Poddar MK: In vivo distribution of lithium in plasma and brain. Ind J Exp Biol 27:950-954, 1989

Godfrey PP, McCkye SJ, White AM, Wood AJ, Grahame-Smith DG: Subacute and chronic in vivo lithium treatment inhibits agonist and fluoride –stimulated inositol phosphate production in rat cortex. J Neurochem 52:498-506, 1989

Godin Y, Heiner L, Mark J, Handel P: Effects of di-n-propylacetate, an anticonvulsant compound, on GABA metabolism. J Neurochem 16:869-873, 1969

Gottesfeld Z: Effect of lithium and other alkali metals on brain chemistry and behavior: glutamic acid and GABA in brain regions. Psychopharmacologia 45:239-242, 1976

Greil W, Steber R, van Calker D: The agonist-stimulated accumulation of inositol phosphates is attenuated in neutrophils from malepatients under chronic lithium therapy. Biol Psychiatry 30:443-451, 1991

Gyulai L, Bolinger L, Leigh JS Jr, Barlow C, Chance B: Phosphorylethanolamine--the major constituent of the phosphomonoester peak observed by 31P-NMR on developing dog brain. FEBS Lett 178:137-142, 1984

Hallcher LM and Sherman WR: The effects of lithium ion and other agents on the activity of myo-inositol-1-phosphatase from bovine brain. J Biol Chem 255:10896-10901, 1980

Hirvonen MR, Cerebral lithium, inositol and inositol monophosphates. Pharmacol and Toxicol 69:22-27, 1991

Hirvonen MR, Lihtamo H, Savolainen K: A gas chromatographic method for the determination of inositol monophosphates in rat brain. Neurochem Res 13:957-962, 1988

Hirvonen MR, Savolainen K: Lithium-induced decrease of brain inositol and increase of brain inositol-1-phosphate is transient. Neurochem Res 16:905-911, 1991

Hoebel BG, Hernandez L, Schwartz DH, Mark GP, Hunter GA: Microdialysis studies of brain norepinephrine, serotonin, and dopamine release during ingestive behavior. Theoretical and clinical implications. Ann N Y Acad Sci 575:171-91, 1989

Honchar MP, Vogler GP, Gish BG, Sherman WR: Evidence that phosphoinositide metabolism in rat cerebral cortex stimulated by pilocarpine, physostigmine, and pargyline in vivo is not changed by chronic lithium treatment. J Neurochem 55:1521-1525, 1990

Horstman DA, Takemura H, Putney JW Jr.: Formation and metabolism of [3H]inositol phosphates in AR42J pancreatoma cells. Substance P-induced Ca2+ mobilization in the apparent absence of inositol 1,4,5-trisphosphate 3-kinase activity. J Biol Chem 263:15297-15303, 1988

Huang KP: The mechanism of protein kinase C activation. Trends Neurosci 12:425-432, 1989

Hughes AR, Putney JW Jr.: Metabolism and functions of inositol phosphates. Biofactors 1:117-121, 1988

Iadarola MJ, Gale K, Dissociation between drug-induced increases in nerve terminal and non-terminal pools of GABA in vivo. Mol Cell Biochem 39:305-330, 1979

Jacobs D, Silverstone T: Dextroamphetamine-induced arousal in human subjects as a model for mania. Psychol Med 23:323-329, 1986

Jakobsen SN, Wiborg O: Selective effects of long-term lithium and carbamazepine administration on G-protein subunit expression in rat brain. Brain Res 780:46-55, 1998

Jope RS, Miller JM, Ferraro TN, Hare TA: Chronic lithium treatment and status epilepticus induced by lithium and pilocarpine cause selective changes in amino acid concentrations in rat brain regions. Neurochem Res 14:829-834, 1989

Jope RS, Song L, Li PP, Young LT, Kish SJ, Pacheco MA, Warsh JJ: The phosphoinositide signal transduction system is impaired in bipolar affective disorder brain. J Neurochem 66:2402-2409, 1996

Jope RS, Williams MB, Li X, Song L, Grimes CA, Unlap MT, Pacheco MA: Modulation of neuronal signal transduction systems by lithium, in Bipolar Medications: Mechanisms of Action. Edited by Manji HK, Bowden CL, Belmaker RH, Washington DC, American Psychiatric Press Inc, 2000, pp 179-196

Kaplan HI, Sadock BJ: Synopsis of Psychiatry. Baltimore, Williams and Wilkins, 1998

Karler R, Calder LD, Thai LH, Bedingfield JB: A dopaminergic-glutamatergic basis for the action of amphetamine and cocaine. Brain Res 658:8-14, 1994

Kato T, Shioiri T, Takahashi S, Inubushi T: Measurement of brain phosphinositide metabolism in bipolar patients using *in vivo* ^{31}P-MRS. J Affect Dis 22:185-190, 1991

Kato T, Takahashi S, Shioiri T, Inubushi: Brain phosphorous metabolism in depressive disorders detected by phosphorous-31 magnetic resonance spectroscopy. J Affect Dis 26:223-230, 1992

Kato T, Takahashi S, Shioiri T, Inubushi T: Alterations in brain phosphorous metabolism in bipolar disorder detected by *in vivo* ^{31}P and ^{7}Li magnetic resonance spectroscopy. J Affect Dis 27:53-60, 1993

Kato T, Shioiri T, Murashita J, Hamakawa H, Inubushi T, Takahashi S: Phosphorus-31 magnetic resonance spectroscopy and ventricular enlargement in bipolar disorder. Psychiatry Res Neuroimaging 55:41-50, 1994a

Kato T, Takahashi S, Shioiri T, Murashita J, Hamakawa H, Inubushi T: Reduction of brain phosphocreatine in bipolar II disorder detected by phophorus-31 magnetic resonance spectroscopy. J Affect Dis 31:125-133, 1994b

Kato T, Shioiri T, Murashita J, Hamakawa H, Takahashi Y, Inubushi T, Takahashi S: Lateralized abnormality of high energy phosphate metabolism in the frontal lobes of patients with bipolar disorder detected by phase-encoded ^{31}P-MRS. Psychological Med 25:557-566, 1995

Koreh K, Monaco ME: The relationship of hormone-sensitive and hormone-insensitive phosphatidylinositol tophosphatidylinositol 4,5-bisphosphate in the WRK-1 cell. J Biol Chem 261:88-91, 1986

Kuczenski R, Segal DS: An escalating dose/multiple high-dose binge pattern of amphetamine administration results in differential changes in the extracellular dopamine response profiles in caudate-putamen and nucleus accumbens. J Neurosci 17:4441-4447, 1997

Lambert PA, Carrez G, Borselli S: Le dipropylacetamide dans le traitement de la psychose maniaco-depressive. Encephale 1:25-31, 1975

Lambert PA: Acute and prophylactic therapies of patients with affective disorders using valpromide (dipropylacetamide), in Anticonvulsants in Affective Disorders. Edited by Emrich HM, Okuma T, Muller AA. Amsterdam, Excerpta Medica, pp 33-44, 1984

Lambert PA, Venaud G: Comparative study of valpromide versus lithium in the treatment of affective disorders. Nervuse 5:57-65, 1992

Larsson OM, Gram L, Schousboe I, Schousboe A: Differential effects of gamma-vinyl GABA and valproate on GABA-transaminase from cultured neurons and astrocytes. Neuropharmacology 25:617-625, 1986

Lesch KP, Aulakh CS, Tolliver TJ, Hill JL, Murphy DL: Regulation of G proteins by chronic antidepressant drug treatment in rat brain: tricyclics but not clorgyline increase Go alpha subunits. Eur J Pharmacol 207:361-364, 1991

Li R, Wing LL, Wyatt RJ, Kirch DG: Effects of haloperidol, lithium, and valproate on phosphoinositide turnover in rat brain. Pharmacol Biochem Behav 46:323-329, 1993

Levine J, Rapaport A, Lev L, Bersudsky Y, Kofman O, Belmaker RH, Shapiro J, Agam G: Inositol treatment raises CSF inositol levels. Brain Res 627:168-170, 1993

Löscher W: GABA in plasma, CSF, and brain of dogs during chronic and acute treatment with γ-acetylenic GABA and valproic acid, in Problems in GABA Research-From Bacteria to Brain. Edited by Okada Y and Roberts E. Amsterdam, Excerpta Medica, 1982, pp 102-109

Löscher W: Effect of inhibitors of GABA transaminase on the synthesis, binding, uptake, and metabolism of GABA. J Neurochem 34:1603-1608, 1980.

Löscher W: Valproate induced changes in GABA metabolism at the subcellular level. Biochem Pharmacol 30:1364-1366, 1981a

Löscher W: Effect of inhibitors of GABA aminotransferase on the metabolism of GABA in brain tissue and synaptosomal fractions. J Neurochem 36:1521-1527, 1981b

Löscher W: In vivo administration of valproate reduces the nerve terminal (synaptosomal) activity of GABA aminotransferase in discrete brain areas of rats. Neurosci Lett 160:177-180, 1993

Löscher W: Valproate: A reappraisal of its pharmacodynamic properties and mechanisms of action. Prog Neurobiol 58:31-59, 1999

Löscher W, Hörstermann D: Differential effects of vigabatrin, gamma-acetylenic GABA, aminooxyacetic acid, and valproate on levels of various amino acids in rat brain regions and plasma. Naunyn-Schmiedeberg's Arch Pharmacol 349:270-278, 1994

Löscher W, Schmidt D: Increase of human plasma GABA by sodium valproate. Epilepsia 21:611-615, 1980

Löscher W, Schmidt D: Plasma GABA levels in neurological treatment of patients under treatment with sodium valproate. Life Sci. 28:2383-2388, 1981

Löscher W, Siemes H: Valproic acid increases γ-aminobutyric acid in CSF of epileptic children. Lancet 2:225, 1984

Löscher W, Siemes H: Cerebrospinal fluid γ-aminobutyric acid levels in children with different types of epilepsy: effect of anticonvulsant treatments. Epilepsia 36:314-319, 1985

Löscher W, Vetter M, Böhme G, Stoltenburg-Didinger G: *In vivo* effects of anticonvulsant drugs on nerve terminals (synaptosomal) GABA levels in 11 brain regions of the rat. J Neural Transmission 63:157-167, 1985

Lubrich B, Patishi Y, Kofman O, Agam G, Berger M, Belmaker RH, van Calker D: Lithium-induced inositol depletion in rat brain after chronic treatment is restricted to the hypothalamus. Mol Psychiatry 2:407-412, 1997

Maitre M, Ciesielski L, Cash C, Mandel P: Comparison of structural characteristics of the 4-aminobutyrate:2-oxoglutarate transaminases from rat and human brain and of their affinities for certain inhibitors. Biochim Biophys Acta 522:385-399, 1978

Manji HK, Chen G, Hsiao, JK, Masana MI, Moore GJ, Potter WZ: Regulation of signal transduction pathways by mood-stabilizing agents: implications for the pathophysiology and treatment of bipolar disorder, in Bipolar Medications: Mechanisms of Action. Edited by Manji HK, Bowden CL, Belmaker RH, Washington DC, American Psychiatric Press Inc, 2000, pp 129-178

Marcus SR, Nadiger HA, Chandrakala MV, Rao TI, Sadasivudu B: Acute and short-term effects of lithium on glutamate metabolism in rat brain. Biochem Pharmacol 35:365-369, 1986

Martin-Gallard A, Rodriguez P, Lopet M, Benavides J, Ugarte M: Effects of dipropylacetate on the glycine clearance enzyme system and glycine levels. Biochem Pharmacol 34:2877-2882, 1985

Moore GJ, Bebchuk JM, Parrish JK, Faulk MW, Arfken CL, Strahl-Bevacqua J, Manji HK: Temporal dissociation between lithium-induced changes in frontal lobe myo-inositol and clinical response in manic-depressive illness. Am J Psychiatry 156:1902-1908, 1999

Moscovich DG, Belmaker RH, Agam G, Livne A: Inositol-1-phosphatase in red blood cells of manic-depressive patients before and during treatment with lithium. Biol Psychiatry 27:552-555, 1990

Naccarato WF, Ray RE, Wells WW: Biosynthesis of myo-inositol in rat mammary gland. Isolation and properties of the enzymes. Arch Biochem Biophys 164:194-201, 1974

Nahorski SR, Kendall DA, Batty I: Receptors and phosphoinositide metabolism in the central nervous system. Biochem Pharmacol. 35:2447-2453, 1986

Nahorski SR, Ragan CI, Challiss RAJ: Lithium and the phosphoinositide cycle: an example of uncompetitive inhibition and its pharmacological consequences. Trends Pharmacol Sci 12:297-303, 1991

Nakano M, Ueda H, Li JY, Matsumoto M, Yanagihara T: Measurement of regional N-acetylaspartate after transient global ischemia in gerbils with or without ischemic tolerance: an index of neuronal survival. Ann Neurol 44:334-340, 1998

O'Donnell T, Rotzinger S, Nakashima TT, Hanstock CC, Ulrich M, Silverstone PH: Chronic lithium and sodium valproate both decrease the concentration of *myo*-inositol and increase the concentration of inositol monophosphates in rat brain. Brain Research 880: 84-91, 2000

Parent MB, Habib MK, Baker GB: Time-dependent changes in brain monoamine oxidase activity and in brain levels of monoamines and amino acids following acute administration of the antidepressant/antipanic drug phenelzine. Biochem Pharmacol 59:1253-63, 2000

Paslawski T, Treit D, Baker GB, George M, Coutts RT: The antidepressant drug phenelzine produces antianxiety effects in the plus-maze and increases in rat brain GABA. Psychopharmacology (Berl) 127:19-24, 1996

Patishi Y, Lubrich B, Berger M, Kofman O, van Calker D, Belmaker RH: Differential uptake of myo-inositol in vivo into rat brain areas. Eur Neuropsychopharmacol 6:73-75, 1996

Patsalos PN, Lascelles PT: Changes in regional brain levels of amino acid putative nurotransmitters after prolonged treatment with the anticonvulsant drugs diphenylhydantoin, phenobarbitone, sodium valproate, ethosuximide, and sulthiame in the rat. J Neurochem 36:688-695, 1981

Peng, L, Hertz, L Huang R, Sonnewald U, Petersen SB, Westergaard N, Larsson O, Schousboe A: Utilization of glutamine and of TCA cycle constituents as precursors for transmitter glutamate and GABA. Dev Neurosci 15:367-377, 1993

Petty F, Rush AJ, Davis JM, Calabrese JR, Kimmel SE, Kramer GL, Small JG, Miller HJ, Swann AE, Orsulak PJ, Blake ME, Bowden CL, Plasma GABA predicts acute response to divalproex in mania. Biol Psychiatry 39:278-284, 1996

Phillips NI, Fowler LJ: The effects of sodium valproate on γ-aminobutyrate metabolism and behavior in naïve and ethanolamine-*O*-sulphate pretreated rats and mice. Biochem Pharmacol 31:2257-2261, 1982

Plenge P: Acute lithium effects on rat brain glucose metabolism-in vivo. Int Pharmacopsychiatry 260:587-596, 1976

Portilla D, Morrison AR: Bradykinin-induced changes in inositol trisphosphate mass in MDCK cells. Biochem Biophys Res Commun 140:644-649, 1986

Post, RM: Approaches to treatment-resistant bipolar affectively ill patients. Clin Neuropharmacol 11:93-114, 1998

Preece NE, Gadian DG, Houseman J, Williams SR: Lithium-induced modulation of cerebral inositol phosphate metabolism in the rat: a multinuclear magnetic resonance study in vivo. Lithium 3:287-297, 1992

Reid MS, Hsu K Jr, Berger SP: Cocaine and amphetamine preferentially stimulate glutamate release in the limbic system: studies on the involvement of dopamine. Synapse 27:95-105, 1997

Rowley HL, Marsden CA, Martin KF: Differential effects of phenytoin and sodium valproate on seizure-induced changes in gamma-aminobutyric acid and glutamate release in vivo. Eur J Pharmacol 294:541-546, 1995

Savolainen KM, Hirvonen MR, Tarhanen J, Nelson SR, Samson FE, Pazdernik TL: Changes in cerebral inositol-1-phosphate concentrations in LiCl-treated rats: regional and strain differences. Neurochem Res 15:541-545, 1990

Seiden LS, Sabol KE, Ricaurte GA: Amphetamine: effects on catecholamine systems and behavior. Annu Rev Pharmacol Toxicol 33:639-677, 1993

Sharma R, Venkatasubramanian PN, Barany M, Davis JM: Proton magnetic resonance spectroscopy of the brain in schizophrenic and affective patients. Schizophr Res 8:43-49, 1992

Shayman JA, Wu D: Myo-inositol does not modulate PI turnover in MDCK cells under hyperosmolar conditions. Am J Physiol 258:F1282-1287, 1990

Sherman R, Leavitt AL, Honchar MP, Hallcher LM, Phillips BE: Evidence that lithium alters phosphoinositide metabolism: chronic administration elevates primarily D-myo-inositol-1-phosphate in cerebral cortex of the rat. J Neurochem 36:1947-1951, 1981

Sherman WR, Munsell LY, Gish BG, Honchar MP: Effects of systematically administered lithium on phosphoinositide metabolism in rat brain, kidney, and testis. J Neurochem 44:798-807, 1985

Shimon H, Agam G, Belmaker RH, Hyde TM, Kleinman JE: Reduced frontal cortex inositol levels in postmortem brain of suicide victims and patients with bipolar disorder. Am J Psychiatry 154:1148-1150, 1997

Silverstone PH, Hanstock CC, Fabian J, Staab R, Allen PS: Chronic lithium does not alter human myo-inositol or phosphomonoester concentrations as measured by 1H and 31P MRS. Biol Psychiatry 40:235-246, 1996

Silverstone PH, Pukhovsky A, Rotzinger S: Lithium does not attenuate the effects of D-amphetamine in healthy volunteers. Psychiatry Res 79:219-226, 1998

Silverstone PH, Rotzinger S, Pukhovsky A, Hanstock CC: Effects of lithium and amphetamine on inositol metabolism in the human brain as measured by 1H and 31P MRS. Biol Psychiatry 46:1634-1641, 1999

Simler S, Randrianarisoa H, Lehman A, Mandel P: Effects du di-n-propylacétate sur les crises audiogènes de la souris. J Physiol (Paris) 60:547, 1968

Singh AK: Quantitative analysis of inositol lipids and inositol phosphates in synaptosomes and microvessels by column chromatography: comparison of the mass analysis and the radiolabelling methods. J Chromatogr 581:1-10, 1992

Singh AK, Jiang Y: Quantitative chromatographic analysis of inositol phospholipids and related compounds. J Chromatogr B Biomed Appl 671:255-280, 1995

Sloley BD, Kah O, Trudeau VL, Dulka JG, Peter RE: Amino acid neurotransmitters and dopamine in brain and pituitary of the goldfish: involvement in the regulation of gonadotropin secretion. J Neurochem 58:2254-2262, 1992

Song L, Jope RS: Chronic lithium treatment impairs phosphatidylinositol hydrolysis in membranes from rat brain regions. J Neurochem 58:2200-2206, 1992

Spector R, Lorenzo AV: The origin of myo-inositol in brain, cerebrospinal fluid and choroid plexus. J Neurochem 25:353-354, 1975

Stubbs EB Jr, Kelleher JA, Sun GY: Phosphatidylinositol kinase, phosphatidylinositol-4-phosphate kinase and diacylglycerol kinase activities in rat brain subcellular fractions. Biochim Biophys Acta 958:247-254, 1988

Sun GY, Navidi M, Yoa FG, Lin TN, Orth OE, Stubbs EB Jr., MacQuarrie RA: Lithium effects on inositol phospholipids and inositol phosphates:

evaluation of an in vivo model for assessing polyphosphoinositide turnover in brain. J Neurochem 58:290-297, 1992

Tarver AP, King WG, Rittenhouse SE: Inositol 1,4,5-trisphosphate and inositol 1,2-cyclic 4,5-trisphosphate are minor components of total mass of inositol trisphosphate in thrombin-stimulated platelets. Rapid formation of inositol 1,3,4-trisphosphate. J Biol Chem 262:17268-17271, 1987

Vadnal R and Parthasarathy R: Myo-inositol monophosphatase: diverse effects of lithium, carbamazepine, and valproate. Neuropsychopharmacol.12:277-285, 1995

van Vaals JJ, Bergman AH, den Boef JH, van den Boogert HJ, van Gerwen PH: ACE: a single-shot method for water-suppressed localization and editing of spectra, images, and spectroscopic images. Magn Reson Med 19:136-160, 1991

Waagepetersen HS, Sonnewald US, Larsson OM, Schousboe A: A possible role of alanine for ammonia transfer between astrocytes and glutaminergic neurons. J Neurochem 75: 471-479, 2000

Wang HY, Friedman E: Effects of lithium on receptor-mediated activation of G proteins in rat brain cortical membranes. Neuropharmacology 38:403-414, 1999

Whittle SR, Turner AJ: Effects of the anticonvulsant sodium valproate on γ-aminobutyrate and aldehyde metabolism in ox brain. J Neurochem 31:1453-1459, 1978

Whitworth P, Heal DJ, Kendall DA: Chronic lithium treatment enhances inositol polyphosphate formation in mouse brain in vivo. Br J Pharmacol 101:39-44, 1990

Whitworth P, Kendall DA: Effects of lithium on inositol phospholipid hydrolysis and inhibition of dopamine D1 receptor-mediated cyclic AMP formation by carbachol in rat brain slices. J Neurochem 53:536-541, 1989

Wikinski SI, Acosta GB, Rubio MC: Valproic acid differs in its in vitro effects on glutamic acid decarboxylase activity in neonatal and adult rat brain. Gen Pharmac 27:635-663, 1996

Yatham LN, Srisurapanont M, Zis AP, Kusumakar V: Comparative studies of the biological distinction between unipolar and bipolar depressions. Life Sci 61: 1445-1455, 1997

Yue K, Davanzo P, Strober M, Thomas MA: Frontal lobe 1H MR spectroscopy of children with bipolar disorder [abstract], Int Soc Magn Res Med 7th Annual meeting, 1999. Philadelphia, PA; Abstract 1471.

Zimmer R, Teelken AW, Gündürewa M, Rüther E, Cramer H: Effect of sodium valproate on CSF GABA, cAMP, cGMP, and homovanillic acid levels in men. Brain Res Bull 5(Suppl. 2):585-588, 1980

Appendix I

A. Calculation of Brain Metabolite Concentrations Using ^{1}H NMR

A 10ml aliquot of homogenate from each rat brain was centrifuged and separated into organic, and aqueous layers and a protein precipitate (see Methods section for details). Of the aqueous layer, 3.5ml were taken to dryness in a vacuum centrifuge. To quantify concentrations of the metabolites of interest, a measured amount of TSP was added to a bottle D_2O (100ml) at the beginning of the experiment and 0.83ml of D_2O was added to each dried brain sample. Thus, TSP was not carried through the extraction procedure and was simply mixed with compounds of interest when the D_2O/TSP mixture was added to the dried sample.

Calculation of the metabolite concentration in the sample undergoing NMR spectroscopy:

[Metabolite]/[TSP] = (Metabolite signal/proton)/(TSP signal/proton)

[Metabolite] = [1.56mM] x (Metabolite signal/proton)/(TSP signal/proton)

Number of moles of the metabolite in the NMR sample:

Moles of metabolite = [Metabolite] x Volume of D_2O added to dried solid

= [Metabolite] x 0.00083 L

Since only 3.5ml of aqueous layer was mixed with the D_2O/TSP mixture and there was 4.0ml of aqueous layer/g of brain tissue, the calculated metabolite concentration must be adjusted to give the brain concentration rather than the NMR sample concentration.

Brain concentration of a given metabolite

= (mmoles of metabolite x 4ml/g brain tissue)/3.5ml

= (mmoles of metabolite)/(g of brain tissue)

B. Calculation of Brain Metabolite Concentrations Using ^{31}P NMR

Brain concentrations of phosphorus-containing metabolites were calculated using the preceding formulae with the exception that DSS was used as an internal standard at a concentration of 0.98mM. A measured amount of DSS was also added to the bottle of D_2O at the beginning of the experiment.

Appendix II

A. HPLC Calibration Plots for Amino Acid Concentrations

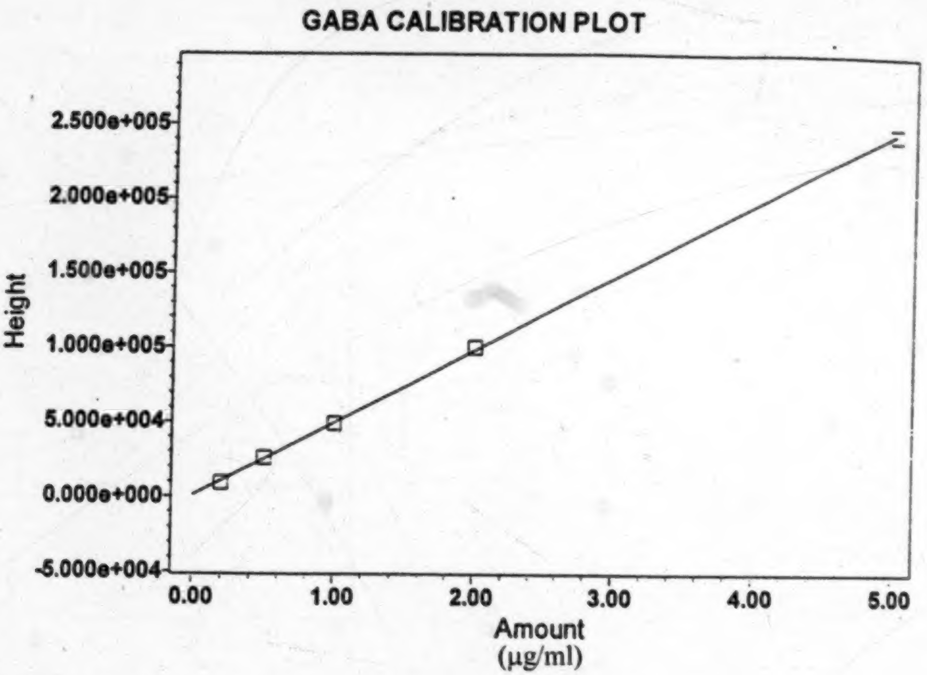

Figure 24: HPLC Calibration Plot for GABA Concentrations. $r^2=0.999816$.

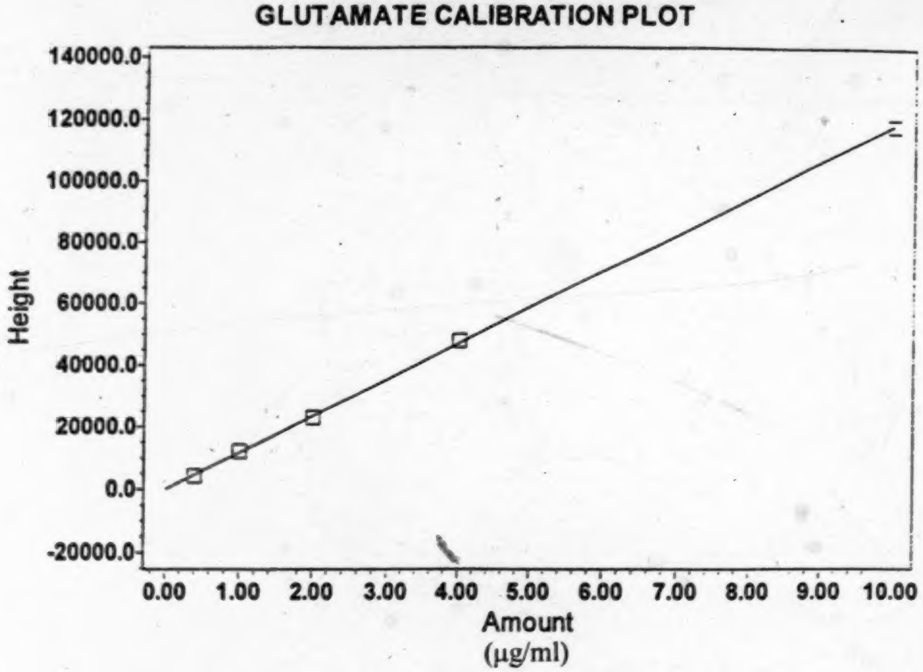

Figure 25: HPLC Calibration Plot for Glutamate Concentrations. r^2*=0.999845.*

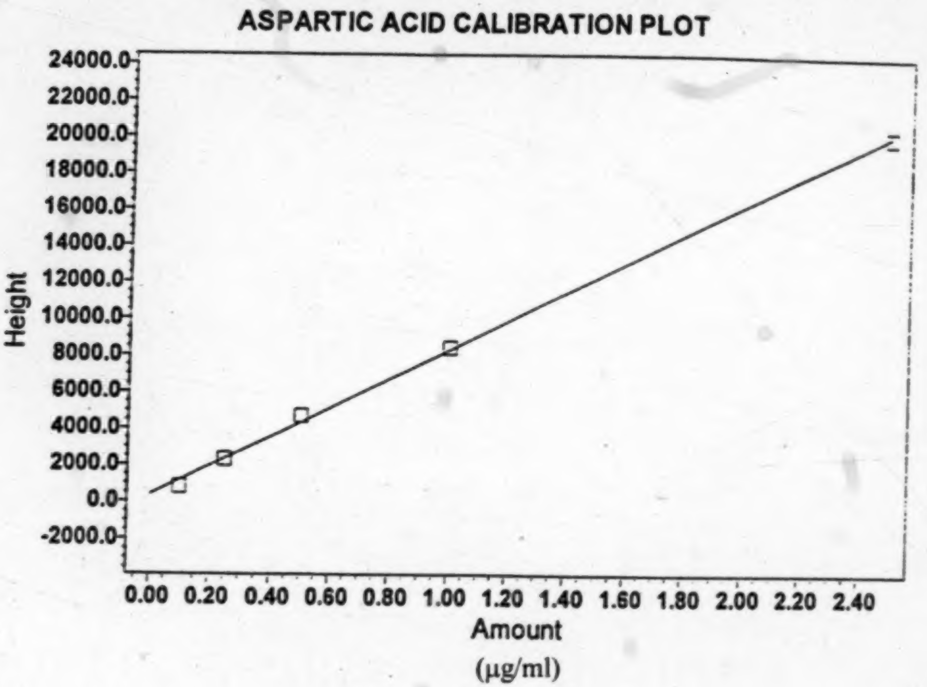

Figure 26: HPLC Calibration Plot for Aspartate Concentrations. r^2*=0.998839.*

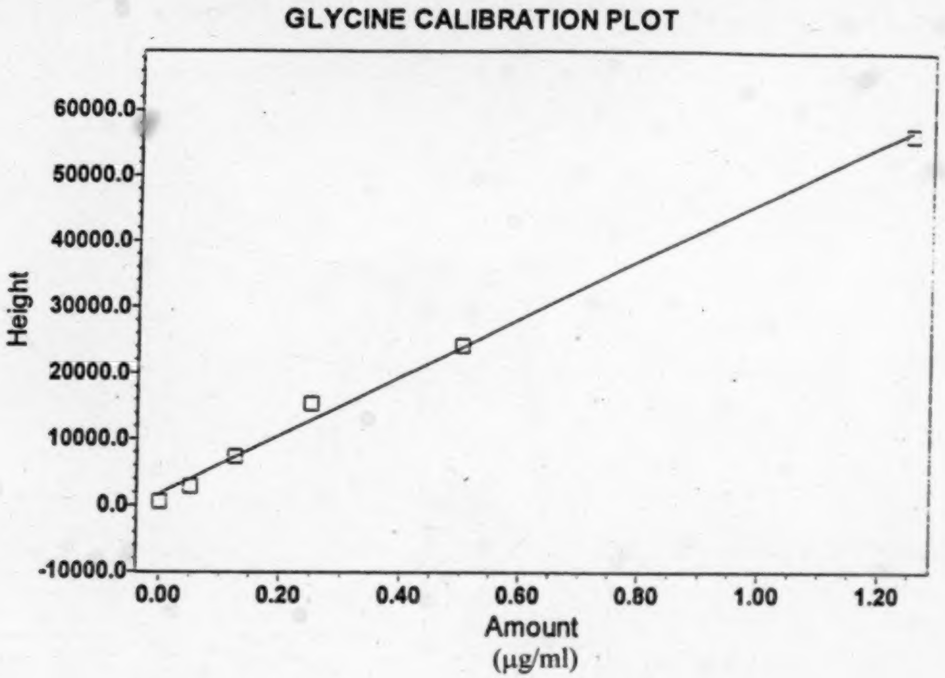

Figure 27: HPLC Calibration Plot for Glycine Concentrations. $r^2=0.995578$.

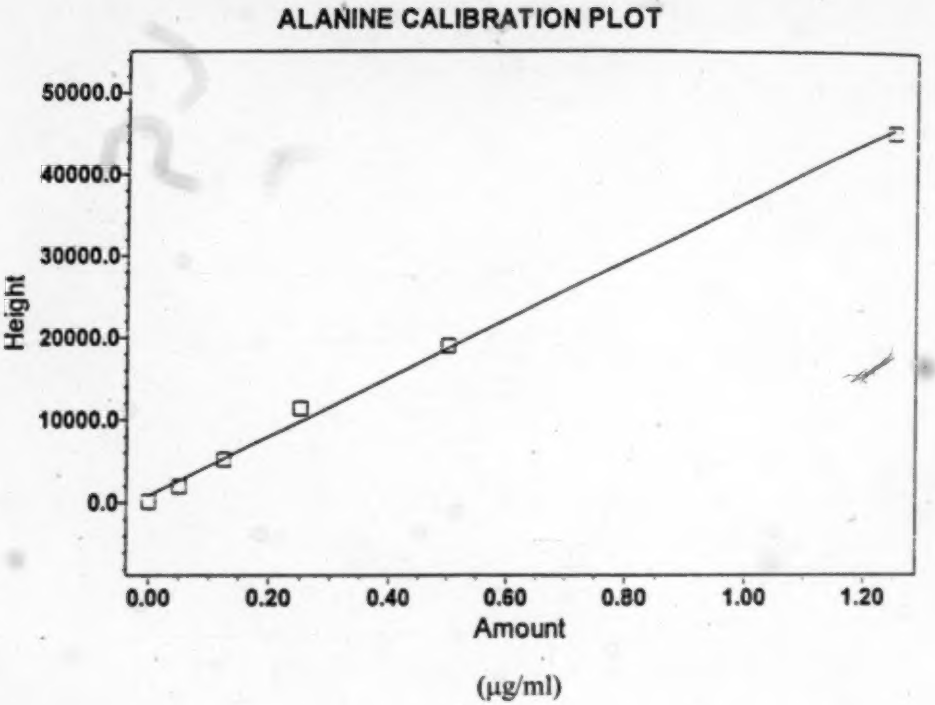

Figure 28: HPLC Calibration Plot for Alanine Concentrations. r^2=0.997204.

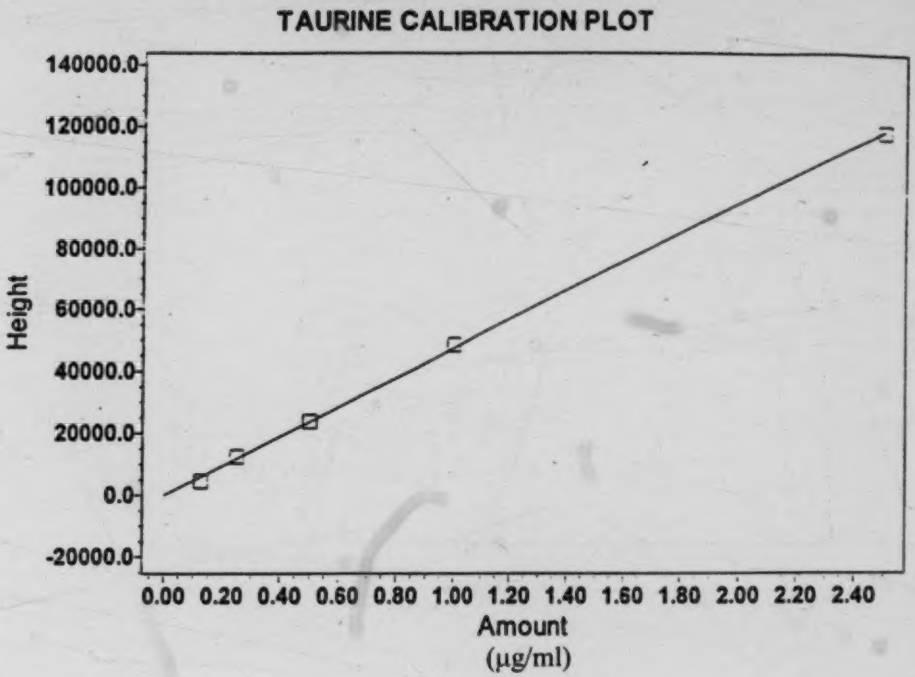

Figure 29: HPLC Calibration Plot for Taurine Concentrations. r^2=0.999557.